FIG. 1

```
TCACGTAAAA AGGGTATCTA GAATTATGAT GATTACTCTG CGCAAACTTC CTCTGGCGGT TGCCGTCGCA GCGGGCGTAA TGTCTGCTCA GGCCATGGCC
AGTGCATTTT TCCCATAGAT CTTAATACTA CTAATGAGAC GCGTTTGAAG GAGACCGCCA ACGGCAGCGT CGCCCGCATT ACAGACGAGT CCGGTACCGG
                         MetMe tIleThrLeu ArgLysLeuP roLeuAlaVa lAlaValAla AlaGlyValM etSerAlaGl nAlaMetAla
                         ^Start of lamB signal sequence

GGTCCCGAAA CTCTGTGCGG TGCTGAACTG GTTGACGCTC TGCAGTTCGT ATGTGGTGAT CGAGGCTTCC TGTTCAACAA ACCGACTGGG GCTGGATCCT
CCAGGGCTTT GAGACACGCC ACGACTTGAC CAACTGCGAG ACGTCAAGCA TACACCACTA GCTCCGAAGG ACAAGTTGTT TGGCTGACCC CGACCTAGGA
GlyProGluT hrLeuCysGl yAlaGluLeu ValAspAlaL euGlnPheVa lCysGlyAsp ArgGlyPheL euPheAsnLy sProThrGly AlaGlySerSer
^Start of IGF-I (Y24L,Y31A)

CCTCTCGTCG TGCTCCCCAG ACTGGTATTG TTGACGAATG CTGCTTTCGT TCTTGCGACC TGCGTCGTCT GGAAATGTAT TGCGCTCCCC TGAAACCCGC
GGAGAGCAGC ACGAGGGGTC TGACCATAAC AACTGCTTAC GACGAAAGCA AGAACGCTGG ACGCAGCAGA CCTTTACATA ACGCGAGGGG ACTTTGGGCG
  SerArgAr gAlaProGln ThrGlyIleV alAspGluCy sCysPheArg SerCysAspL euArgArgLe uGluMetTyr CysAlaProL euLysProAla

TAAATCTGCT TAGAAGCTCC TAACGCTCGG TTGCCGCCGG GCGTTTTTTA TTGTTAACTC ATGTTTGACA GCTTATCATC GATAAGCTTT AATGCGGTAG
ATTTAGACGA ATCTTCGAGG ATTGCGAGCC AACGGCGGCC CGCAAAAAAT AACAATTGAG TACAAACTGT CGAATAGTAG CTATTCGAAA TTACGCCATC
 LysSerAla Am*
```

Figure 1: Nucleotide and Amino Acid Sequence of the LamB Signal Sequence and IGF-I (Y24L,Y31A)

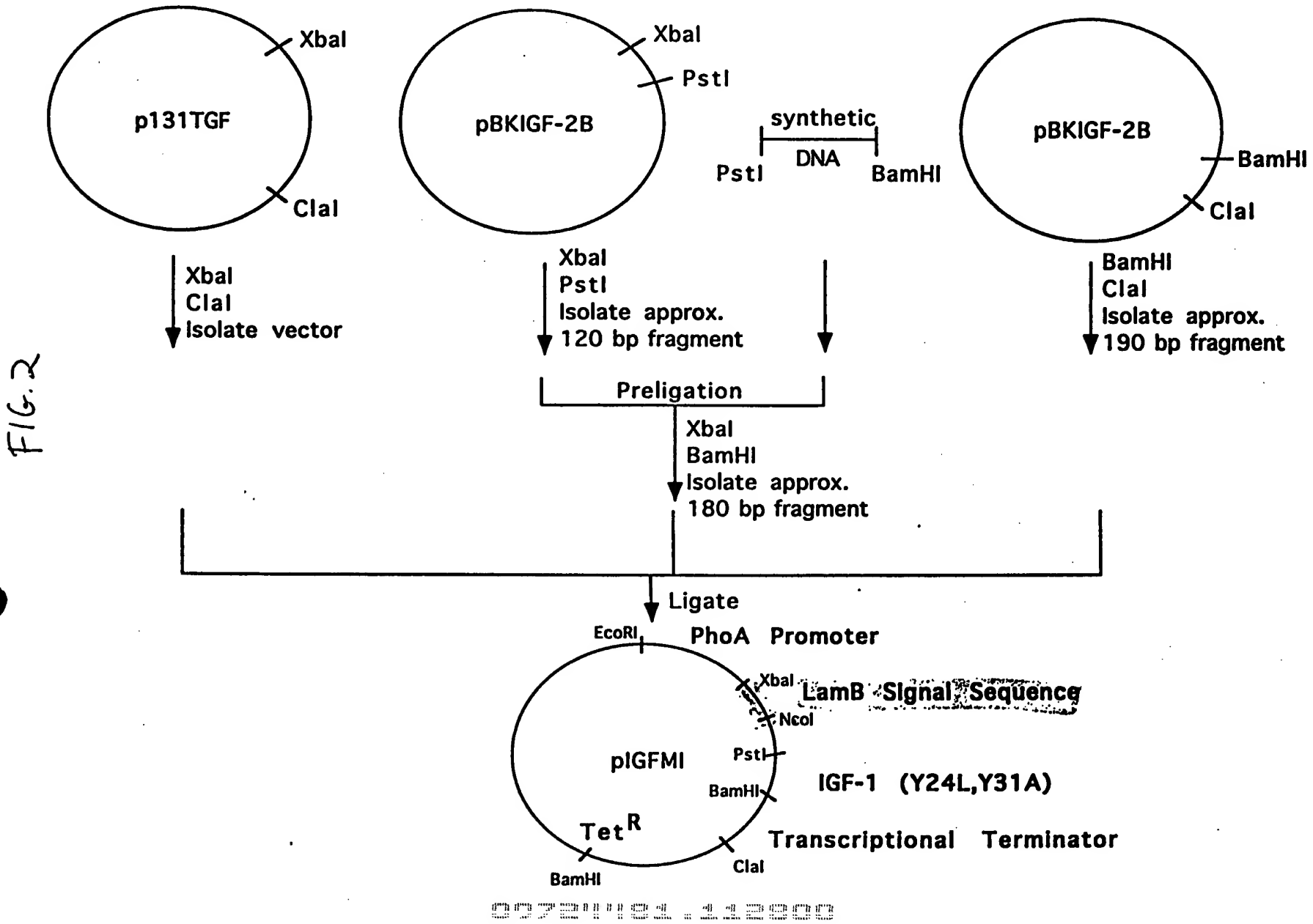
p131TGF
XbaI
ClaI
pBKIGF-2B
XbaI
PstI
synthetic
DNA
PstI
BamHI
pBKIGF-2B
BamHI
ClaI
XbaI
ClaI
Isolate vector
XbaI
PstI
Isolate approx.
120 bp fragment
BamHI
ClaI
Isolate approx.
190 bp fragment
Preligation
XbaI
BamHI
Isolate approx.
180 bp fragment
Ligate
EcoRI
PhoA Promoter
XbaI
LamB Signal Sequence
NcoI
pIGFMI
PstI
BamHI
IGF-1 (Y24L,Y31A)
Tet R
Transcriptional Terminator
BamHI
ClaI

FIG. 2

FIGURE 3

plasmid IGFMI
length: 5115 (circular)

```
   1 GAATTCAACT TCTCCATACT TTGGATAAGG AAATACAGAC ATGAAAAATC TCATTGCTGA GTTGTTATTT AAGCTTGCCC AAAAAGAAGA AGAGTCGAAT
     CTTAAGTTGA AGAGGTATGA AACCTATTCC TTTATGTCTG TACTTTTTAG AGTAACGACT CAACAATAAA TTCGAACGGG TTTTTCTTCT TCTCAGCTTA

 101 GAACTGTGTG CGCAGGTAGA AGCTTTGGAG ATTATCGTCA CTGCAATGCT TCGCAATATG GCGCAAAATG ACCAACAGCG GTTGATTGAT CAGGTAGAGG
     CTTGACACAC GCGTCCATCT TCGAAACCTC TAATAGCAGT GACGTTACGA AGCGTTATAC CGCGTTTTAC TGGTTGTCGC CAACTAACTA GTCCATCTCC

 201 GGGCGCTGTA CGAGGTAAAG CCCGATGCCA GCATTCCTGA CGACGATACG GAGCTGCTGC GCGATTACGT AAAGAAGTTA TTGAAGCATC CTCGTCAGTA
     CCCGCGACAT GCTCCATTTC GGGCTACGGT CGTAAGGACT GCTGCTATGC CTCGACGACG CGCTAATGCA TTTCTTCAAT AACTTCGTAG GAGCAGTCAT

 301 AAAAGTTAAT CTTTTCAACA GCTGTCATAA AGTTGTCACG GCCGAGACTT ATAGTCGCTT TGTTTTTATT TTTTAATGTA TTTGTAACTA GTACGCAAGT
     TTTTCAATTA GAAAAGTTGT CGACAGTATT TCAACAGTGC CGGCTCTGAA TATCAGCGAA ACAAAAATAA AAAATTACAT AAACATTGAT CATGCGTTCA

 401 TCACGTAAAA AGGGTATCTA GAATTATGAT GATTACTCTG CGCAAACTTC CTCTGGCGGT TGCCGTCGCA GCGGGCGTAA TGTCTGCTCA GGCCATGGCC
     AGTGCATTTT TCCCATAGAT CTTAATACTA CTAATGAGAC GCGTTTGAAG GAGACCGCCA ACGGCAGCGT CGCCCGCATT ACAGACGAGT CCGGTACCGG
   1                          MetMe tIleThrLeu ArgLysLeuP roLeuAlaVa lAlaValAla AlaGlyValM etSerAlaGl nAlaMetAla

 501 GGTCCCGAAA CTCTGTGCGG TGCTGAACTG GTTGACGCTC TGCAGTTCGT ATGTGGTGAT CGAGGCTTCC TGTTCAACAA ACCGACTGGG GCTGGATCCT
     CCAGGGCTTT GAGACACGCC ACGACTTGAC CAACTGCGAG ACGTCAAGCA TACACCACTA GCTCCGAAGG ACAAGTTGTT TGGCTGACCC CGACCTAGGA
  26 GlyProGluT hrLeuCysGl yAlaGluLeu ValAspAlaL euGlnPheVa lCysGlyAsp ArgGlyPheL euPheAsnLy sProThrGly AlaGlySerSer

 601 CCTCTCGTCG TGCTCCCCAG ACTGGTATTG TTGACGAATG CTGCTTTCGT TCTTGCGACC TGCGTCGTCT GGAAATGTAT TGCGCTCCCC TGAAACCCGC
     GGAGAGCAGC ACGAGGGGTC TGACCATAAC AACTGCTTAC GACGAAAGCA AGAACGCTGG ACGCAGCAGA CCTTTACATA ACGCGAGGGG ACTTTGGGCG
  60   SerArgAr gAlaProGln ThrGlyIleV alAspGluCy sCysPheArg SerCysAspL euArgArgLe uGluMetTyr CysAlaProL euLysProAla

 701 TAAATCTGCT TAGAAGCTCC TAACGCTCGG TTGCCGCCGG GCGTTTTTTA TTGTTAACTC ATGTTTGACA GCTTATCATC GATAAGCTTT AATGCGGTAG
     ATTTAGACGA ATCTTCGAGG ATTGCGAGCC AACGGCGGCC CGCAAAAAAT AACAATTGAG TACAAACTGT CGAATAGTAG CTATTCGAAA TTACGCCATC
  93  LysSerAla Am*

 801 TTTATCACAG TTAAATTGCT AACGCAGTCA GGCACCGTGT ATGAAATCTA ACAATGCGCT CATCGTCATC CTCGGCACCG TCACCCTGGA TGCTGTAGGC
     AAATAGTGTC AATTTAACGA TTGCGTCAGT CCGTGGCACA TACTTTAGAT TGTTACGCGA GTAGCAGTAG GAGCCGTGGC AGTGGGACCT ACGACATCCG

 901 ATAGGCTTGG TTATGCCGGT ACTGCCGGGC CTCTTGCGGG ATATCGTCCA TTCCGACAGC ATCGCCAGTC ACTATGGCGT GCTGCTAGCG CTATATGCGT
     TATCCGAACC AATACGGCCA TGACGGCCCG GAGAACGCCC TATAGCAGGT AAGGCTGTCG TAGCGGTCAG TGATACCGCA CGACGATCGC GATATACGCA

1001 TGATGCAATT TCTATGCGCA CCCGTTCTCG GAGCACTGTC CGACCGCTTT GGCCGCCGCC CAGTCCTGCT CGCTTCGCTA CTTGGAGCCA CTATCGACTA
     ACTACGTTAA AGATACGCGT GGGCAAGAGC CTCGTGACAG GCTGGCGAAA CCGGCGGCGG GTCAGGACGA GCGAAGCGAT GAACCTCGGT GATAGCTGAT

1101 CGCGATCATG GCGACCACAC CCGTCCTGTG GATCCTCTAC GCCGGACGCA TCGTGGCCGG CATCACCGGC GCCACAGGTG CGGTTGCTGG CGCCTATATC
     GCGCTAGTAC CGCTGGTGTG GGCAGGACAC CTAGGAGATG CGGCCTGCGT AGCACCGGCC GTAGTGGCCG CGGTGTCCAC GCCAACGACC GCGGATATAG

1201 GCCGACATCA CCGATGGGGA AGATCGGGCT CGCCACTTCG GGCTCATGAG CGCTTGTTTC GGCGTGGGTA TGGTGGCAGG CCCCGTGGCC GGGGGACTGT
     CGGCTGTAGT GGCTACCCCT TCTAGCCCGA GCGGTGAAGC CCGAGTACTC GCGAACAAAG CCGCACCCAT ACCACCGTCC GGGGCACCGG CCCCCTGACA
```

FIGURE 3 (cont'd)

```
1301 TGGGCGCCAT CTCCTTGCAT GCACCATTCC TTGCGGCGGC GGTGCTCAAC GGCCTCAACC TACTACTGGG CTGCTTCCTA ATGCAGGAGT CGCATAAGGG
     ACCCGCGGTA GAGGAACGTA CGTGGTAAGG AACGCCGCCG CCACGAGTTG CCGGAGTTGG ATGATGACCC GACGAAGGAT TACGTCCTCA GCGTATTCCC

1401 AGAGCGTCGA CCGATGCCCT TGAGAGCCTT CAACCCAGTC AGCTCCTTCC GGTGGGCGCG GGGCATGACT ATCGTCGCCG CACTTATGAC TGTCTTCTTT
     TCTCGCAGCT GGCTACGGGA ACTCTCGGAA GTTGGGTCAG TCGAGGAAGG CCACCCGCGC CCCGTACTGA TAGCAGCGGC GTGAATACTG ACAGAAGAAA

1501 ATCATGCAAC TCGTAGGACA GGTGCCGGCA GCGCTCTGGG TCATTTTCGG CGAGGACCGC TTTCGCTGGA GCGCGACGAT GATCGGCCTG TCGCTTGCGG
     TAGTACGTTG AGCATCCTGT CCACGGCCGT CGCGAGACCC AGTAAAAGCC GCTCCTGGCG AAAGCGACCT CGCGCTGCTA CTAGCCGGAC AGCGAACGCC

1601 TATTCGGAAT CTTGCACGCC CTCGCTCAAG CCTTCGTCAC TGGTCCCGCC ACCAAACGTT TCGGCGAGAA GCAGGCCATT ATCGCCGGCA TGGCGGCCGA
     ATAAGCCTTA GAACGTGCGG GAGCGAGTTC GGAAGCAGTG ACCAGGGCGG TGGTTTGCAA AGCCGCTCTT CGTCCGGTAA TAGCGGCCGT ACCGCCGGCT

1701 CGCGCTGGGC TACGTCTTGC TGGCGTTCGC GACGCGAGGC TGGATGGCCT TCCCCATTAT GATTCTTCTC GCTTCCGGCG GCATCGGGAT GCCCGCGTTG
     GCGCGACCCG ATGCAGAACG ACCGCAAGCG CTGCGCTCCG ACCTACCGGA AGGGGTAATA CTAAGAAGAG CGAAGGCCGC CGTAGCCCTA CGGGCGCAAC

1801 CAGGCCATGC TGTCCAGGCA GGTAGATGAC GACCATCAGG GACAGCTTCA AGGATCGCTC GCGGCTCTTA CCAGCCTAAC TTCGATCACT GGACCGCTGA
     GTCCGGTACG ACAGGTCCGT CCATCTACTG CTGGTAGTCC CTGTCGAAGT TCCTAGCGAG CGCCGAGAAT GGTCGGATTG AAGCTAGTGA CCTGGCGACT

1901 TCGTCACGGC GATTTATGCC GCCTCGGCGA GCACATGGAA CGGGTTGGCA TGGATTGTAG GCGCCGCCCT ATACCTTGTC TGCCTCCCCG CGTTGCGTCG
     AGCAGTGCCG CTAAATACGG CGGAGCCGCT CGTGTACCTT GCCCAACCGT ACCTAACATC CGCGGCGGGA TATGGAACAG ACGGAGGGGC GCAACGCAGC

2001 CGGTGCATGG AGCCGGGCCA CCTCGACCTG AATGGAAGCC GGCGGCACCT CGCTAACGGA TTCACCACTC CAAGAATTGG AGCCAATCAA TTCTTGCGGA
     GCCACGTACC TCGGCCCGGT GGAGCTGGAC TTACCTTCGG CCGCCGTGGA GCGATTGCCT AAGTGGTGAG GTTCTTAACC TCGGTTAGTT AAGAACGCCT

2101 GAACTGTGAA TGCGCAAACC AACCCTTGGC AGAACATATC CATCGCGTCC GCCATCTCCA GCAGCCGCAC GCGGCGCATC TCGGGCAGCG TTGGGTCCTG
     CTTGACACTT ACGCGTTTGG TTGGGAACCG TCTTGTATAG GTAGCGCAGG CGGTAGAGGT CGTCGGCGTG CGCCGCGTAG AGCCCGTCGC AACCCAGGAC

2201 GCCACGGGTG CGCATGATCG TGCTCCTGTC GTTGAGGACC CGGCTAGGCT GGCGGGGTTG CCTTACTGGT TAGCAGAATG AATCACCGAT ACGCGAGCGA
     CGGTGCCCAC GCGTACTAGC ACGAGGACAG CAACTCCTGG GCCGATCCGA CCGCCCCAAC GGAATGACCA ATCGTCTTAC TTAGTGGCTA TGCGCTCGCT

2301 ACGTGAAGCG ACTGCTGCTG CAAAACGTCT GCGACCTGAG CAACAACATG AATGGTCTTC GGTTTCCGTG TTTCGTAAAG TCTGGAAACG CGGAAGTCAG
     TGCACTTCGC TGACGACGAC GTTTTGCAGA CGCTGGACTC GTTGTTGTAC TTACCAGAAG CCAAAGGCAC AAAGCATTTC AGACCTTTGC GCCTTCAGTC

2401 CGCCCTGCAC CATTATGTTC CGGATCTGCA TCGCAGGATG CTGCTGGCTA CCCTGTGGAA CACCTACATC TGTATTAACG AAGCGCTGGC ATTGACCCTG
     GCGGGACGTG GTAATACAAG GCCTAGACGT AGCGTCCTAC GACGACCGAT GGGACACCTT GTGGATGTAG ACATAATTGC TTCGCGACCG TAACTGGGAC

2501 AGTGATTTTT CTCTGGTCCC GCCGCATCCA TACCGCCAGT TGTTTACCCT CACAACGTTC CAGTAACCGG GCATGTTCAT CATCAGTAAC CCGTATCGTG
     TCACTAAAAA GAGACCAGGG CGGCGTAGGT ATGGCGGTCA ACAAATGGGA GTGTTGCAAG GTCATTGGCC CGTACAAGTA GTAGTCATTG GGCATAGCAC

2601 AGCATCCTCT CTCGTTTCAT CGGTATCATT ACCCCCATGA ACAGAAATTC CCCCTTACAC GGAGGCATCA AGTGACCAAA CAGGAAAAAA CCGCCCTTAA
     TCGTAGGAGA GAGCAAAGTA GCCATAGTAA TGGGGGTACT TGTCTTTAAG GGGGAATGTG CCTCCGTAGT TCACTGGTTT GTCCTTTTTT GGCGGGAATT

2701 CATGGCCCGC TTTATCAGAA GCCAGACATT AACGCTTCTG GAGAAACTCA ACGAGCTGGA CGCGGATGAA CAGGCAGACA TCTGTGAATC GCTTCACGAC
     GTACCGGGCG AAATAGTCTT CGGTCTGTAA TTGCGAAGAC CTCTTTGAGT TGCTCGACCT GCGCCTACTT GTCCGTCTGT AGACACTTAG CGAAGTGCTG

2801 CACGCTGATG AGCTTTACCG CAGCTGCCTC GCGCGTTTCG GTGATGACGG TGAAAACCTC TGACACATGC AGCTCCCGGA GACGGTCACA GCTTGTCTGT
     GTGCGACTAC TCGAAATGGC GTCGACGGAG CGCGCAAAGC CACTACTGCC ACTTTTGGAG ACTGTGTACG TCGAGGGCCT CTGCCAGTGT CGAACAGACA

2901 AAGCGGATGC CGGGAGCAGA CAAGCCCGTC AGGGCGCGTC AGCGGGTGTT GGCGGGTGTC GGGGCGCAGC CATGACCCAG TCACGTAGCG ATAGCGGAGT
     TTCGCCTACG GCCCTCGTCT GTTCGGGCAG TCCCGCGCAG TCGCCCACAA CCGCCCACAG CCCCGCGTCG GTACTGGGTC AGTGCATCGC TATCGCCTCA
```

FIGURE 3 (cont'd)

3001 GTATACTGGC TTAACTATGC GGCATCAGAG CAGATTGTAC TGAGAGTGCA CCATATGCGG TGTGAAATAC CGCACAGATG CGTAAGGAGA AAATACCGCA
CATATGACCG AATTGATACG CCGTAGTCTC GTCTAACATG ACTCTCACGT GGTATACGCC ACACTTTATG GCGTGTCTAC GCATTCCTCT TTTATGGCGT

3101 TCAGGCGCTC TTCCGCTTCC TCGCTCACTG ACTCGCTGCG CTCGGTCGTT CGGCTGCGGC GAGCGGTATC AGCTCACTCA AAGGCGGTAA TACGGTTATC
AGTCCGCGAG AAGGCGAAGG AGCGAGTGAC TGAGCGACGC GAGCCAGCAA GCCGACGCCG CTCGCCATAG TCGAGTGAGT TTCCGCCATT ATGCCAATAG

3201 CACAGAATCA GGGGATAACG CAGGAAAGAA CATGTGAGCA AAAGGCCAGC AAAAGGCCAG GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG
GTGTCTTAGT CCCCTATTGC GTCCTTTCTT GTACACTCGT TTTCCGGTCG TTTTCCGGTC CTTGGCATTT TTCCGGCGCA ACGACCGCAA AAAGGTATCC

3301 CTCCGCCCCC CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT AAAGATACCA GGCGTTTCCC CCTGGAAGCT
GAGGCGGGGG GACTGCTCGT AGTGTTTTTA GCTGCGAGTT CAGTCTCCAC CGCTTTGGGC TGTCCTGATA TTTCTATGGT CCGCAAAGGG GGACCTTCGA

3401 CCCTCGTGCG CTCTCCTGTT CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC CTTCGGGAAG CGTGGCGCTT TCTCATAGCT CACGCTGTAG
GGGAGCACGC GAGAGGACAA GGCTGGGACG GCGAATGGCC TATGGACAGG CGGAAAGAGG GAAGCCCTTC GCACCGCGAA AGAGTATCGA GTGCGACATC

3501 GTATCTCAGT TCGGTGTAGG TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT TCAGCCCGAC CGCTGCGCCT TATCCGGTAA CTATCGTCTT
CATAGAGTCA AGCCACATCC AGCAAGCGAG GTTCGACCCG ACACACGTGC TTGGGGGGCA AGTCGGGCTG GCGACGCGGA ATAGGCCATT GATAGCAGAA

3601 GAGTCCAACC CGGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA
CTCAGGTTGG GCCATTCTGT GCTGAATAGC GGTGACCGTC GTCGGTGACC ATTGTCCTAA TCGTCTCGCT CCATACATCC GCCACGATGT CTCAAGAACT

3701 AGTGGTGGCC TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC CTTCGGAAAA AGAGTTGGTA GCTCTTGATC
TCACCACCGG ATTGATGCCG ATGTGATCTT CCTGTCATAA ACCATAGACG CGAGACGACT TCGGTCAATG GAAGCCTTTT TCTCAACCAT CGAGAACTAG

3801 CGGCAAACAA ACCACCGCTG GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGCG CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT
GCCGTTTGTT TGGTGGCGAC CATCGCCACC AAAAAAACAA ACGTTCGTCG TCTAATGCGC GTCTTTTTTT CCTAGAGTTC TTCTAGGAAA CTAGAAAAGA

3901 ACGGGGTCTG ACGCTCAGTG GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA TCAAAAAGGA TCTTCACCTA GATCCTTTTA AATTAAAAAT
TGCCCCAGAC TGCGAGTCAC CTTGCTTTTG AGTGCAATTC CCTAAAACCA GTACTCTAAT AGTTTTTCCT AGAAGTGGAT CTAGGAAAAT TTAATTTTTA

4001 GAAGTTTTAA ATCAATCTAA AGTATATATG AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA GGCACCTATC TCAGCGATCT GTCTATTTCG
CTTCAAAATT TAGTTAGATT TCATATATAC TCATTTGAAC CAGACTGTCA ATGGTTACGA ATTAGTCACT CCGTGGATAG AGTCGCTAGA CAGATAAAGC

4101 TTCATCCATA GTTGCCTGAC TCCCCGTCGT GTAGATAACT ACGATACGGG AGGGCTTACC ATCTGGCCCC AGTGCTGCAA TGATACCGCG AGACCCACGC
AAGTAGGTAT CAACGGACTG AGGGGCAGCA CATCTATTGA TGCTATGCCC TCCCGAATGG TAGACCGGGG TCACGACGTT ACTATGGCGC TCTGGGTGCG

4201 TCACCGGCTC CAGATTTATC AGCAATAAAC CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT GGTCCTGCAA CTTTATCCGC CTCCATCCAG TCTATTAATT
AGTGGCCGAG GTCTAAATAG TCGTTATTTG GTCGGTCGGC CTTCCCGGCT CGCGTCTTCA CCAGGACGTT GAAATAGGCG GAGGTAGGTC AGATAATTAA

4301 GTTGCCGGGA AGCTAGAGTA AGTAGTTCGC CAGTTAATAG TTTGCGCAAC GTTGTTGCCA TTGCTGCAGG CATCGTGGTG TCACGCTCGT CGTTTGGTAT
CAACGGCCCT TCGATCTCAT TCATCAAGCG GTCAATTATC AAACGCGTTG CAACAACGGT AACGACGTCC GTAGCACCAC AGTGCGAGCA GCAAACCATA

4401 GGCTTCATTC AGCTCCGGTT CCCAACGATC AAGGCGAGTT ACATGATCCC CCATGTTGTG CAAAAAAGCG GTTAGCTCCT TCGGTCCTCC GATCGTTGTC
CCGAAGTAAG TCGAGGCCAA GGGTTGCTAG TTCCGCTCAA TGTACTAGGG GGTACAACAC GTTTTTTCGC CAATCGAGGA AGCCAGGAGG CTAGCAACAG

4501 AGAAGTAAGT TGGCCGCAGT GTTATCACTC ATGGTTATGG CAGCACTGCA TAATTCTCTT ACTGTCATGC CATCCGTAAG ATGCTTTTCT GTGACTGGTG
TCTTCATTCA ACCGGCGTCA CAATAGTGAG TACCAATACC GTCGTGACGT ATTAAGAGAA TGACAGTACG GTAGGCATTC TACGAAAAGA CACTGACCAC

4601 AGTACTCAAC CAAGTCATTC TGAGAATAGT GTATGCGGCG ACCGAGTTGC TCTTGCCCGG CGTCAACACG GGATAATACC GCGCCACATA GCAGAACTTT
TCATGAGTTG GTTCAGTAAG ACTCTTATCA CATACGCCGC TGGCTCAACG AGAACGGGCC GCAGTTGTGC CCTATTATGG CGCGGTGTAT CGTCTTGAAA

FIGURE 3 (cont'd)

```
4701 AAAAGTGCTC ATCATTGGAA AACGTTCTTC GGGGCGAAAA CTCTCAAGGA TCTTACCGCT GTTGAGATCC AGTTCGATGT AACCCACTCG TGCACCCAAC
     TTTTCACGAG TAGTAACCTT TTGCAAGAAG CCCCGCTTTT GAGAGTTCCT AGAATGGCGA CAACTCTAGG TCAAGCTACA TTGGGTGAGC ACGTGGGTTG

4801 TGATCTTCAG CATCTTTTAC TTTCACCAGC GTTTCTGGGT GAGCAAAAAC AGGAAGGCAA AATGCCGCAA AAAAGGGAAT AAGGGCGACA CGGAAATGTT
     ACTAGAAGTC GTAGAAAATG AAAGTGGTCG CAAAGACCCA CTCGTTTTTG TCCTTCCGTT TTACGGCGTT TTTTCCCTTA TTCCCGCTGT GCCTTTACAA

4901 GAATACTCAT ACTCTTCCTT TTTCAATATT ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTTGAA TGTATTTAGA AAAATAAACA
     CTTATGAGTA TGAGAAGGAA AAAGTTATAA TAACTTCGTA AATAGTCCCA ATAACAGAGT ACTCGCCTAT GTATAAACTT ACATAAATCT TTTTATTTGT

5001 AATAGGGGTT CCGCGCACAT TTCCCCGAAA AGTGCCACCT GACGTCTAAG AAACCATTAT TATCATGACA TTAACCTATA AAAATAGGCG TATCACGAGG
     TTATCCCCAA GGCGCGTGTA AAGGGGCTTT TCACGGTGGA CTGCAGATTC TTTGGTAATA ATAGTACTGT AATTGGATAT TTTTATCCGC ATAGTGCTCC

5101 CCCTTTCGTC TTCAA
     GGGAAAGCAG AAGTT
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FIG. 4

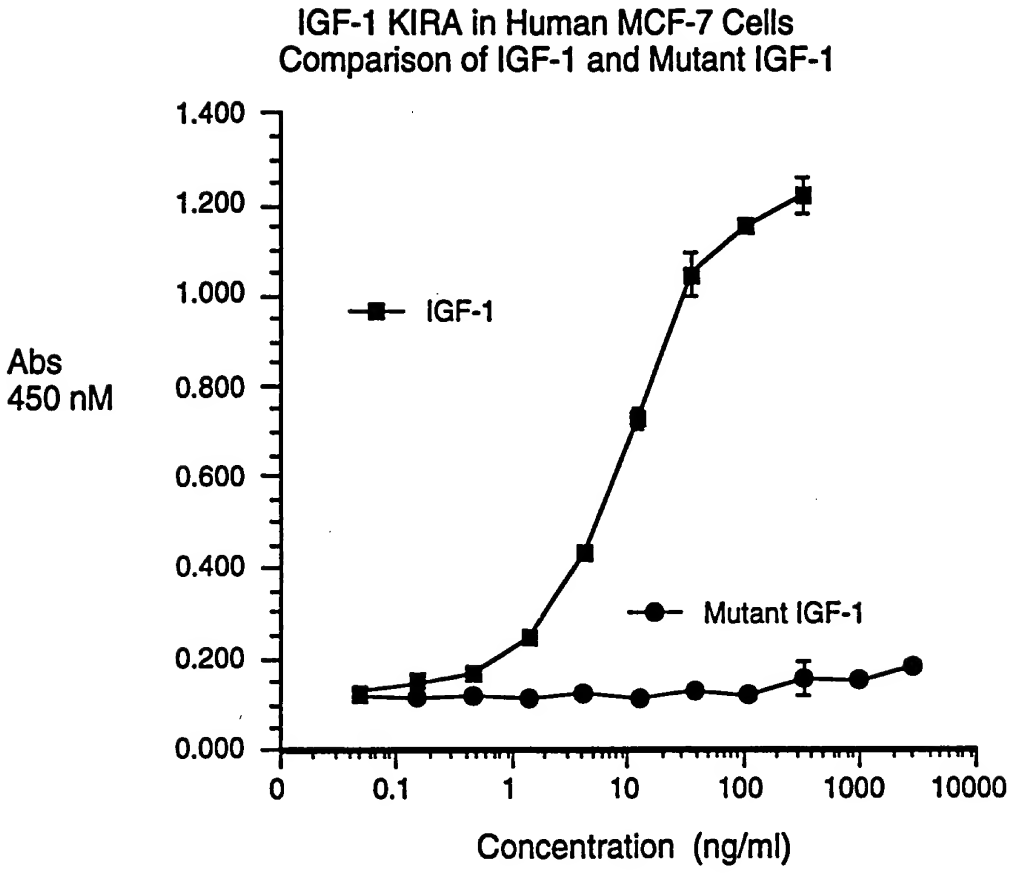

IGF-1 KIRA in Human MCF-7 Cells
Comparison of IGF-1 and Mutant IGF-1
Abs
450 nM
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
IGF-1
Mutant IGF-1
0
0.1
1
10
100
1000
10000
Concentration (ng/ml)

FIG. 3

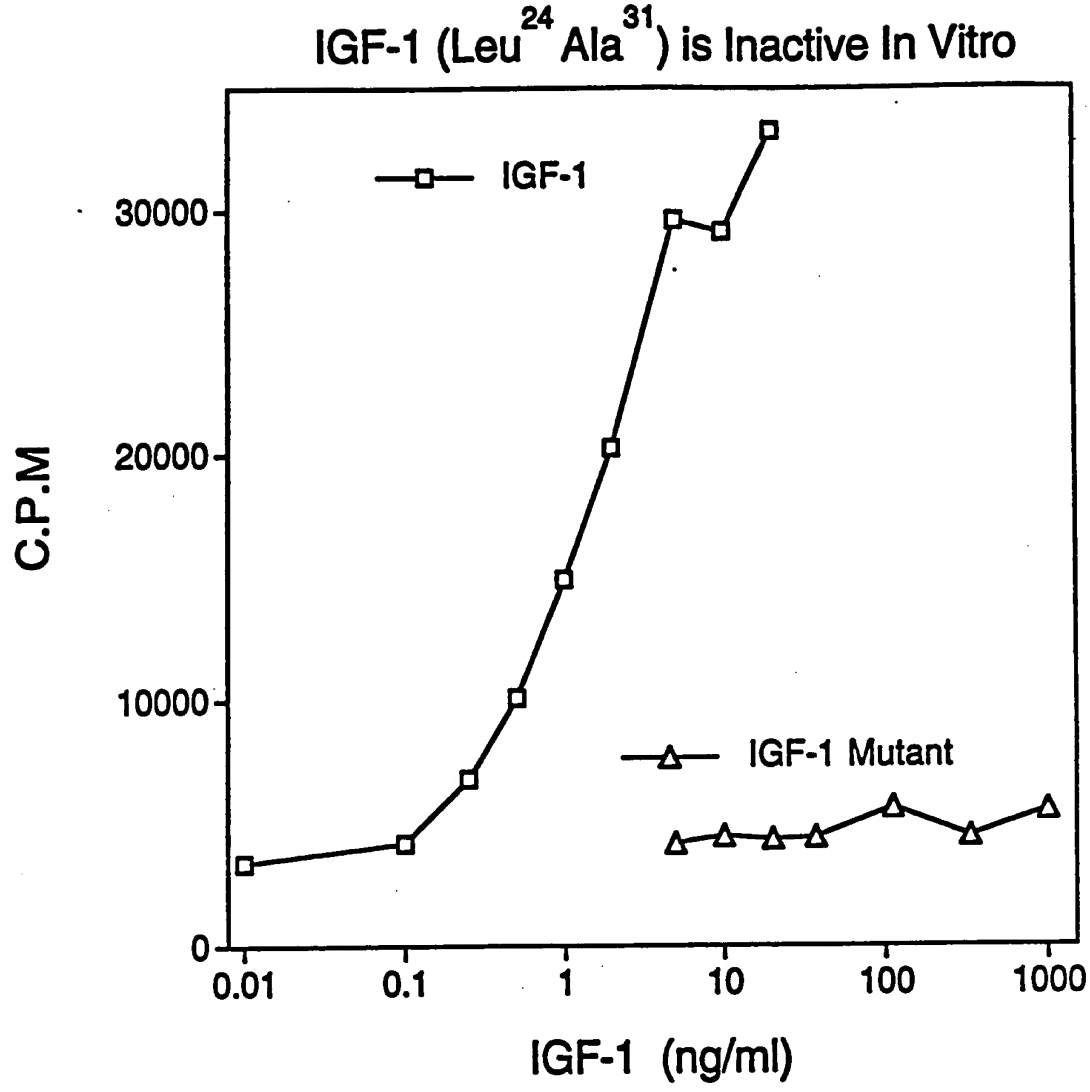

IGF-1 (Leu24 Ala31) is Inactive In Vitro
IGF-1
IGF-1 Mutant
C.P.M
30000
20000
10000
0
0.01
0.1
1
10
100
1000
IGF-1 (ng/ml)

FIG. 6

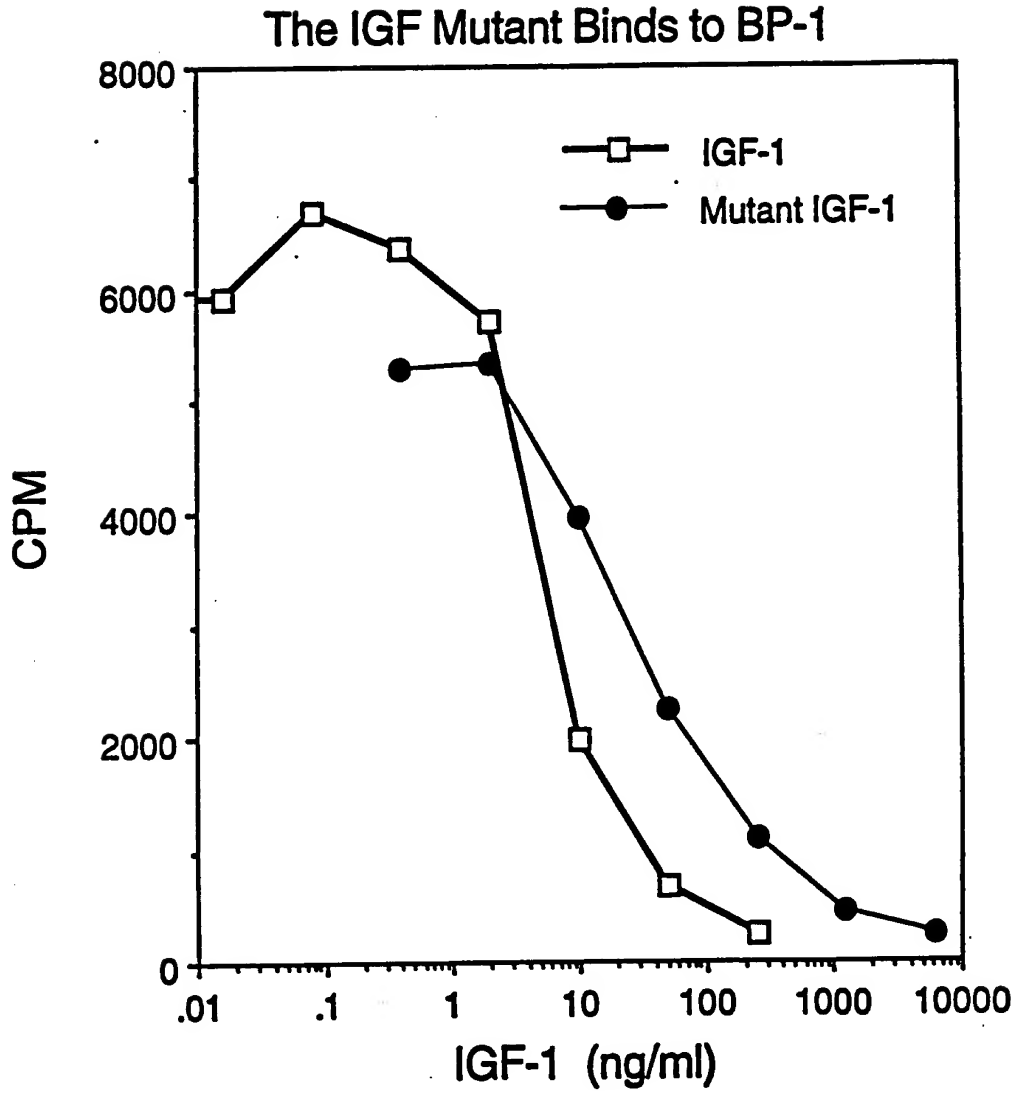

The IGF Mutant Binds to BP-1
8000
6000
4000
2000
0
CPM
IGF-1
Mutant IGF-1
.01
.1
1
10
100
1000
10000
IGF-1 (ng/ml)

FIG. 7

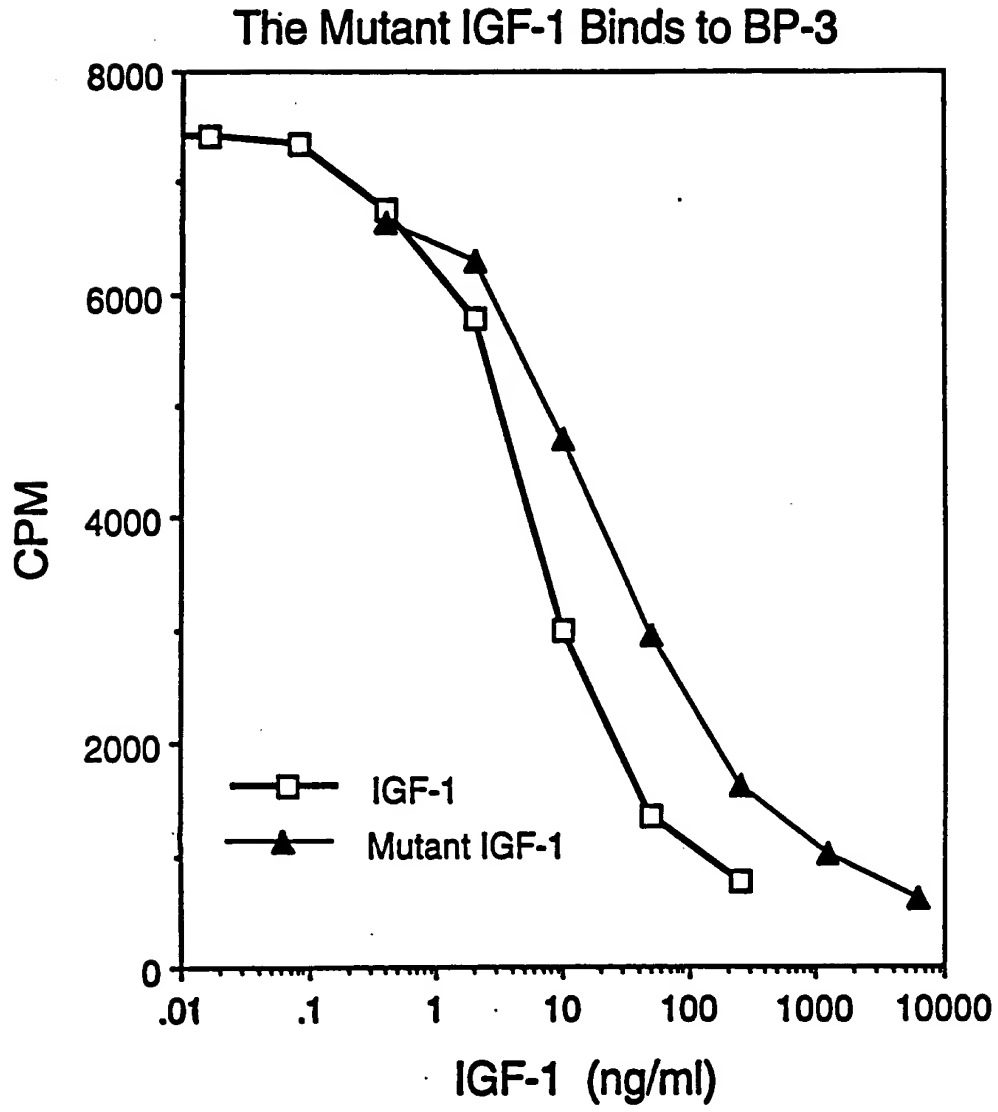

The Mutant IGF-1 Binds to BP-3
8000
6000
4000
2000
0
CPM
IGF-1
Mutant IGF-1
.01
.1
1
10
100
1000
10000
IGF-1 (ng/ml)

FIG. 8

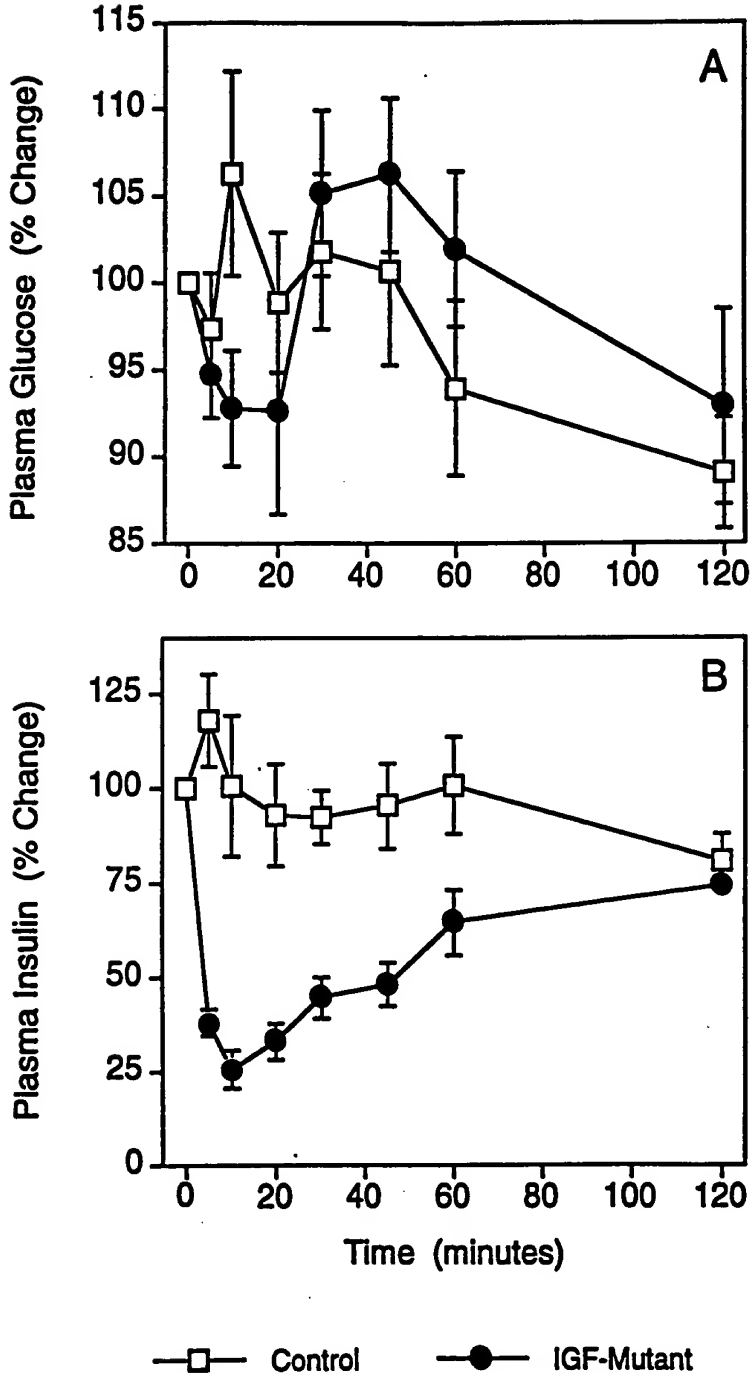

A
B
Plasma Glucose (% Change)
Plasma Insulin (% Change)
115
110
105
100
95
90
85
125
100
75
50
25
0
0
20
40
60
80
100
120
Time (minutes)
Control
IGF-Mutant

FIG. 9

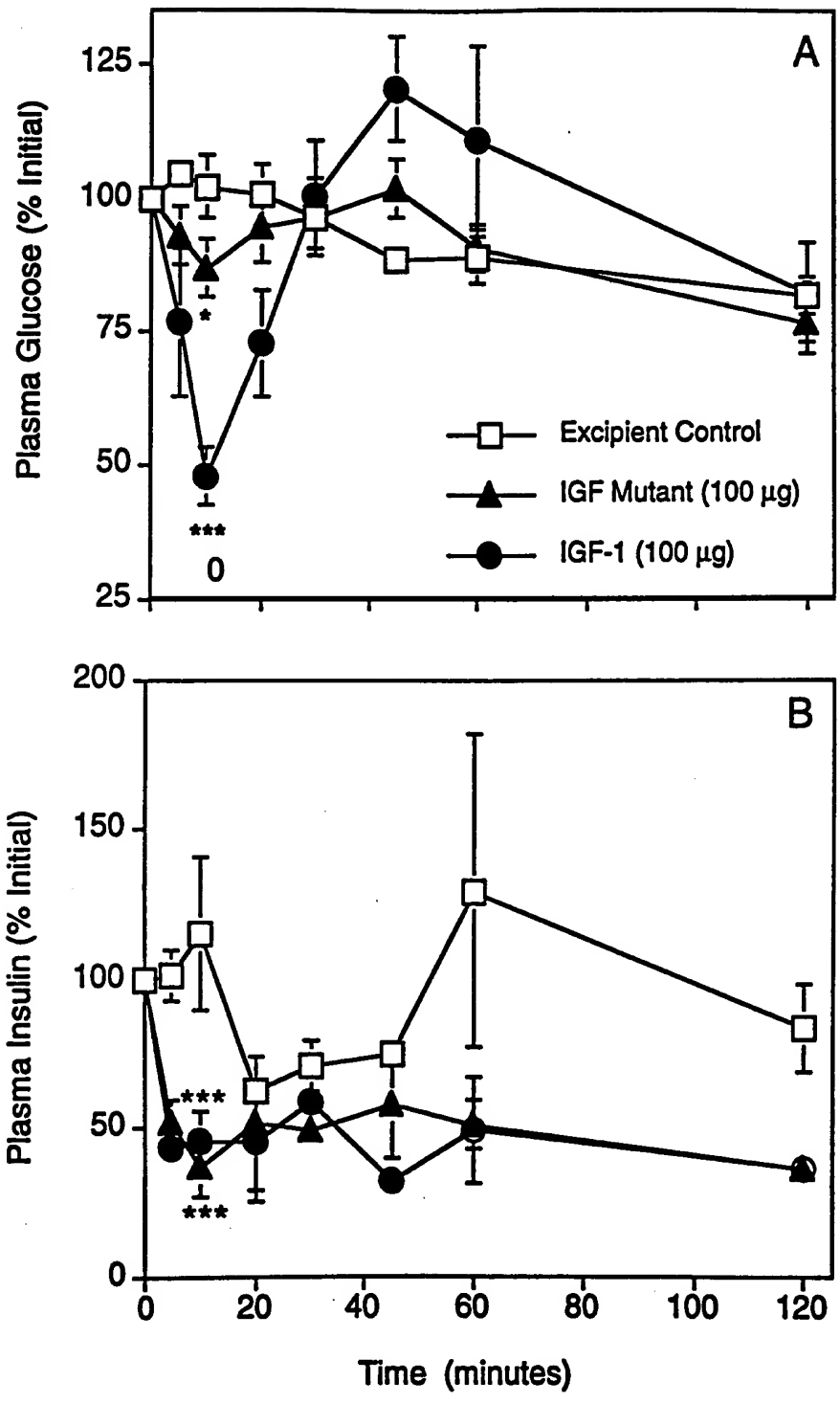

A
B
Plasma Glucose (% Initial)
Plasma Insulin (% Initial)
125
100
75
50
25
200
150
100
50
0
0
20
40
60
80
100
120
Time (minutes)
Excipient Control
IGF Mutant (100 µg)
IGF-1 (100 µg)
*
***
0
***
***

Figure 10

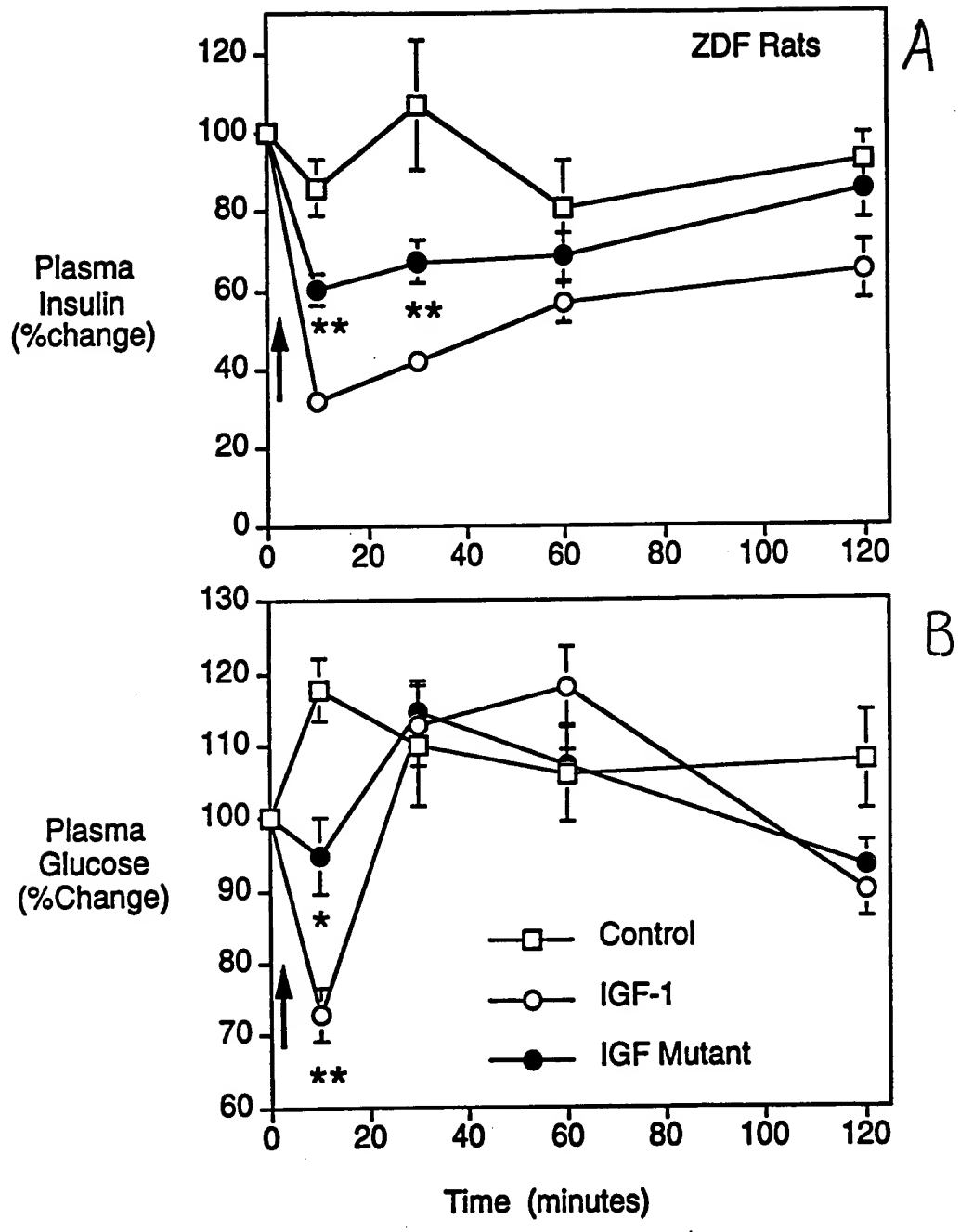

ZDF Rats
A
B
Plasma Insulin (%change)
Plasma Glucose (%Change)
120
100
80
60
40
20
0
130
110
90
70
*
**
Control
IGF-1
IGF Mutant
0
20
40
60
80
100
120
Time (minutes)

FIG. 11

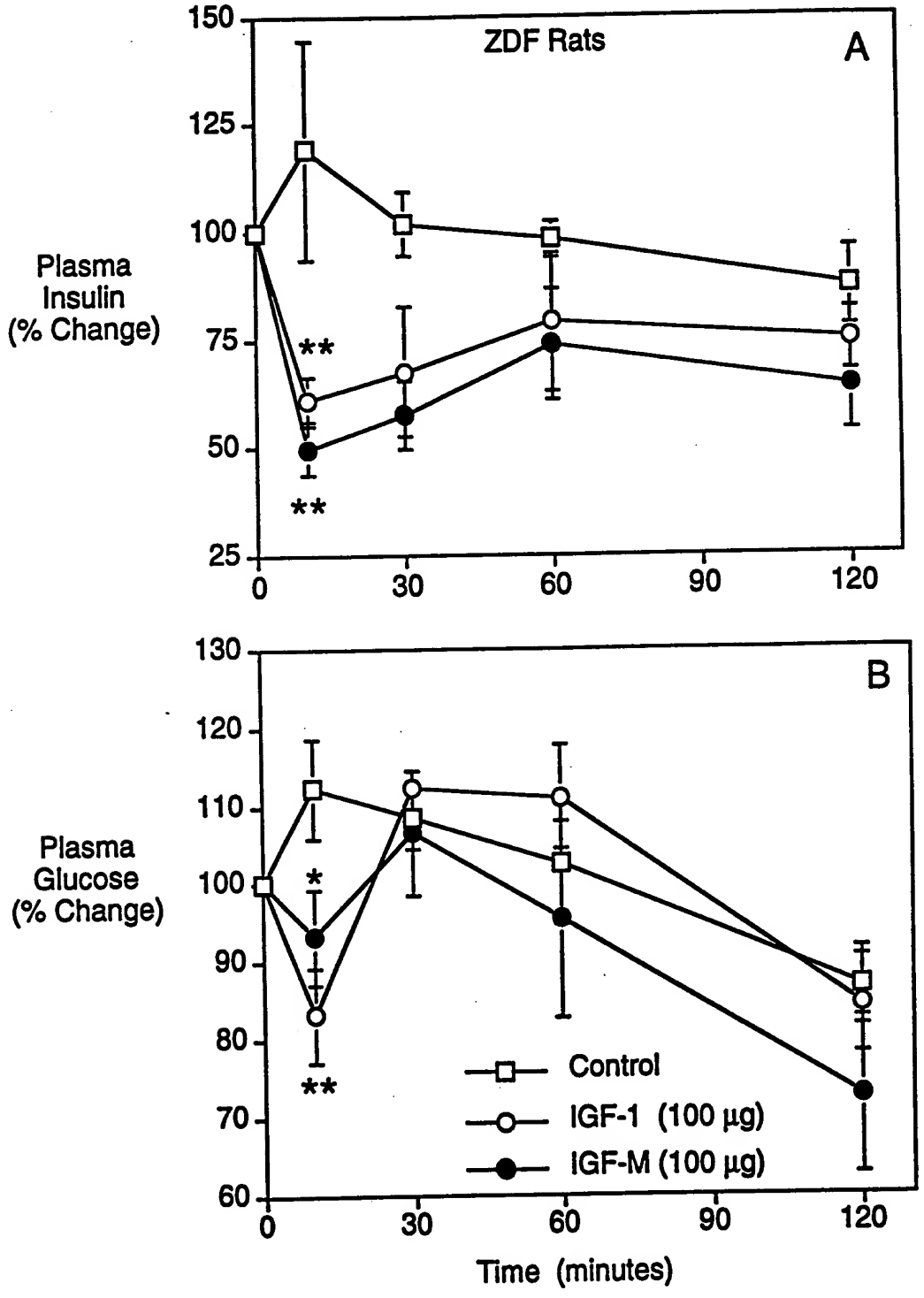

ZDF Rats
A
B
Plasma Insulin (% Change)
Plasma Glucose (% Change)
150
125
100
75
50
25
130
120
110
100
90
80
70
60
0
30
60
90
120
*
**
**
Control
IGF-1 (100 µg)
IGF-M (100 µg)
Time (minutes)

FIG. 12

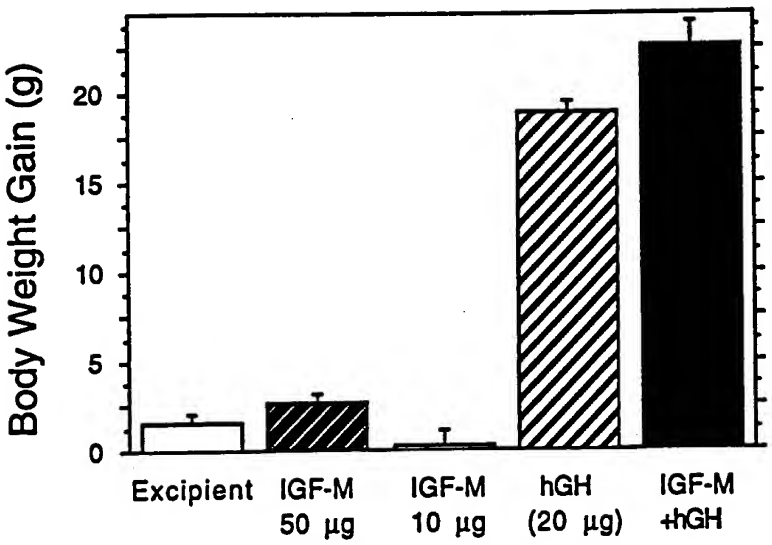

Body Weight Gain (g)
0
5
10
15
20
Excipient
IGF-M 50 µg
IGF-M 10 µg
hGH (20 µg)
IGF-M +hGH

FIG. 13

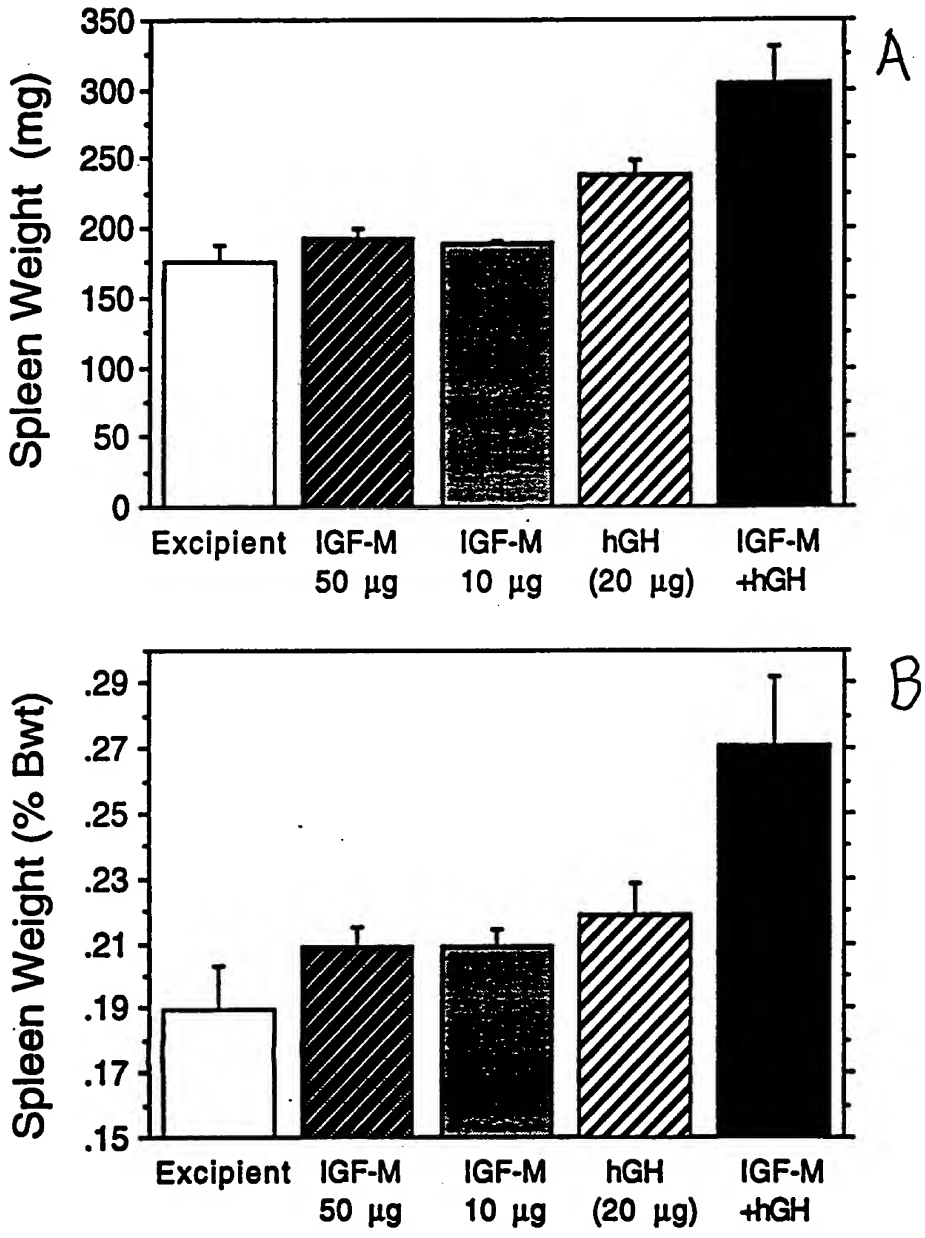
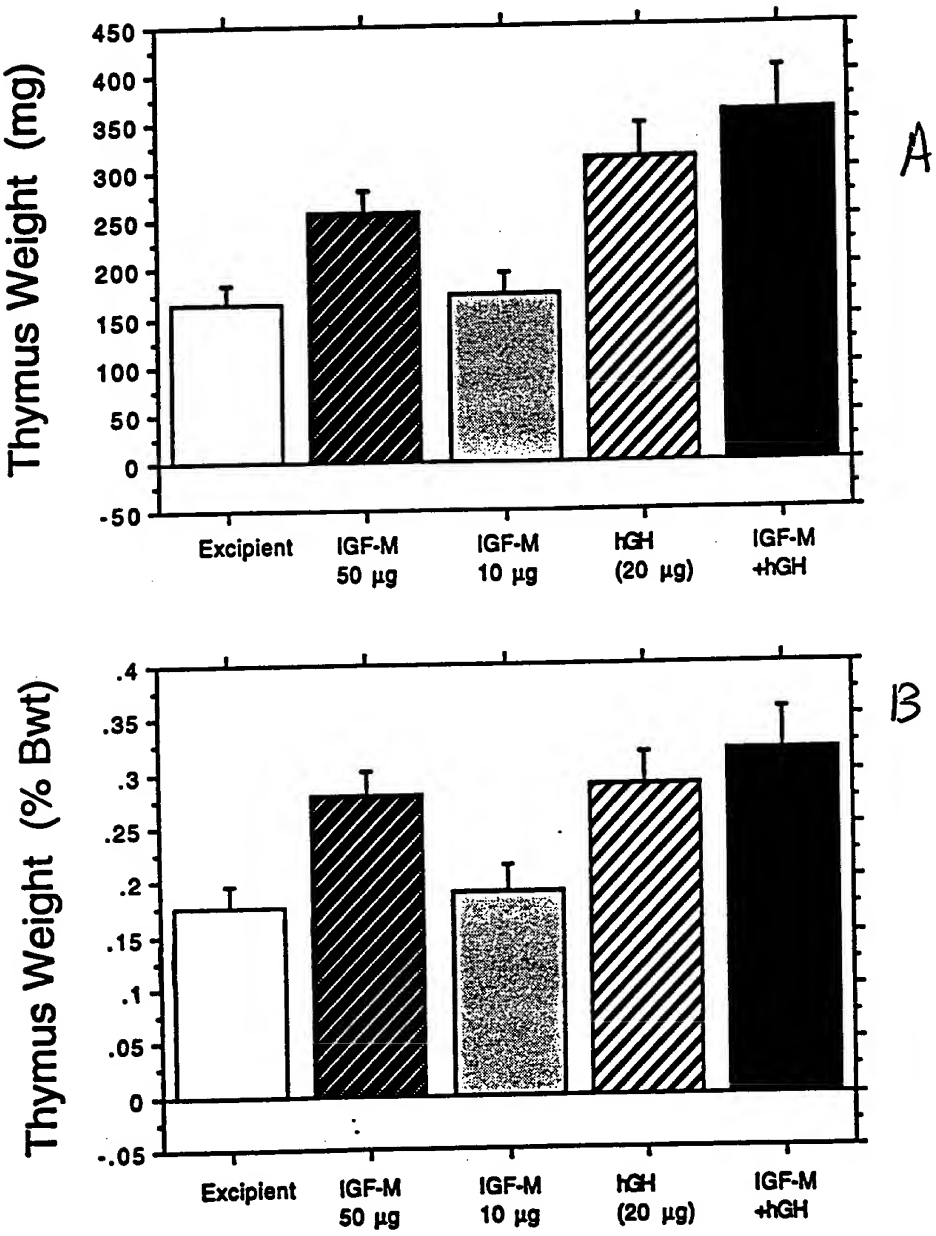

A
Spleen Weight (mg)
350
300
250
200
150
100
50
0
Excipient
IGF-M 50 µg
IGF-M 10 µg
hGH (20 µg)
IGF-M +hGH
B
Spleen Weight (% Bwt)
.29
.27
.25
.23
.21
.19
.17
.15
Excipient
IGF-M 50 µg
IGF-M 10 µg
hGH (20 µg)
IGF-M +hGH

FIG. 14


Thymus Weight (mg)
450
400
350
300
250
200
150
100
50
0
-50
Excipient
IGF-M 50 µg
IGF-M 10 µg
hGH (20 µg)
IGF-M +hGH
A
Thymus Weight (% Bwt)
.4
.35
.3
.25
.2
.15
.1
.05
0
-.05
Excipient
IGF-M 50 µg
IGF-M 10 µg
hGH (20 µg)
IGF-M +hGH
B

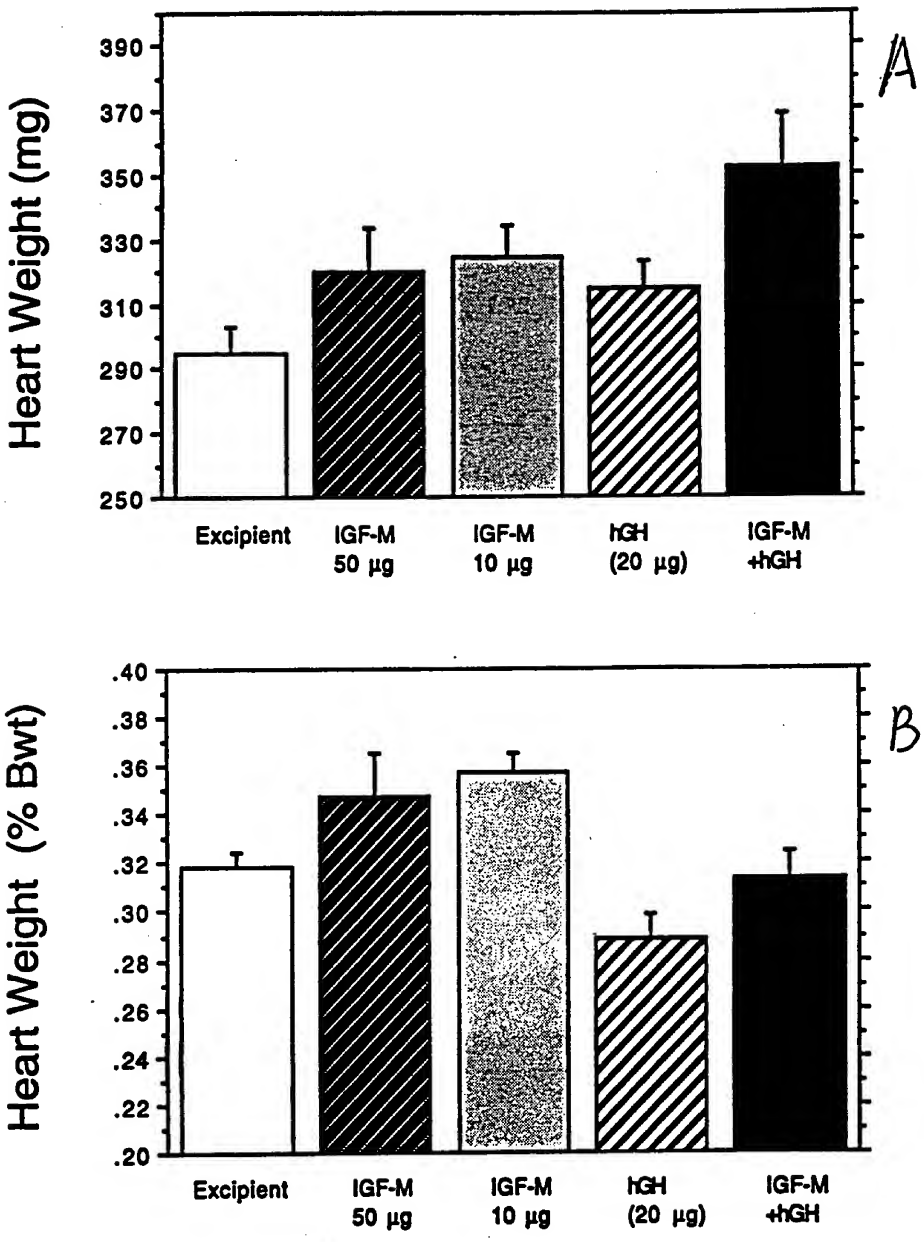

Heart Weight (mg)
390
370
350
330
310
290
270
250
A
Excipient
IGF-M 50 µg
IGF-M 10 µg
hGH (20 µg)
IGF-M +hGH
Heart Weight (% Bwt)
.40
.38
.36
.34
.32
.30
.28
.26
.24
.22
.20
B
Excipient
IGF-M 50 µg
IGF-M 10 µg
hGH (20 µg)
IGF-M +hGH

FIG. 15

FIG. 16

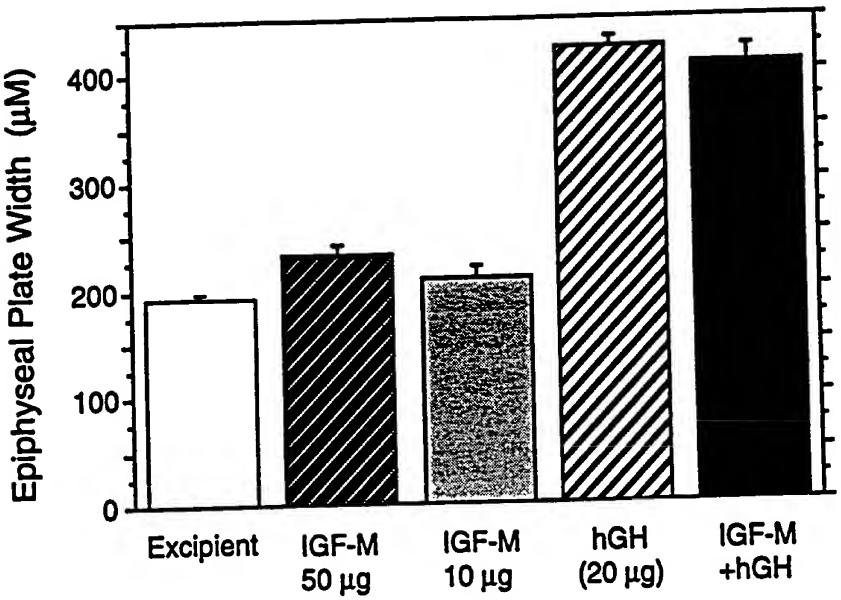

Epiphyseal Plate Width (µM)
400
300
200
100
0
Excipient
IGF-M 50 µg
IGF-M 10 µg
hGH (20 µg)
IGF-M +hGH

FIG. 17

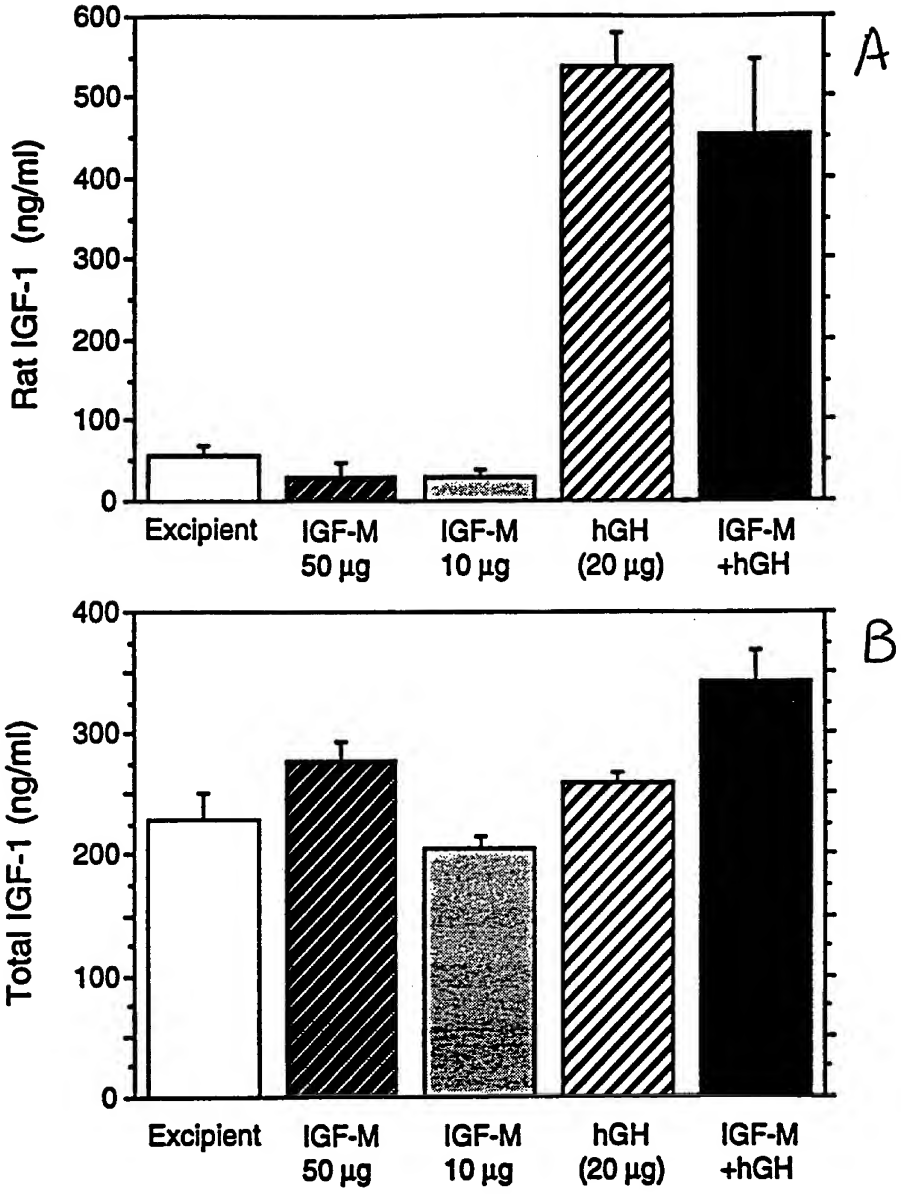

A
Rat IGF-1 (ng/ml)
600
500
400
300
200
100
0
Excipient
IGF-M 50 µg
IGF-M 10 µg
hGH (20 µg)
IGF-M +hGH
B
Total IGF-1 (ng/ml)
400
300
200
100
0
Excipient
IGF-M 50 µg
IGF-M 10 µg
hGH (20 µg)
IGF-M +hGH

FIG. 18

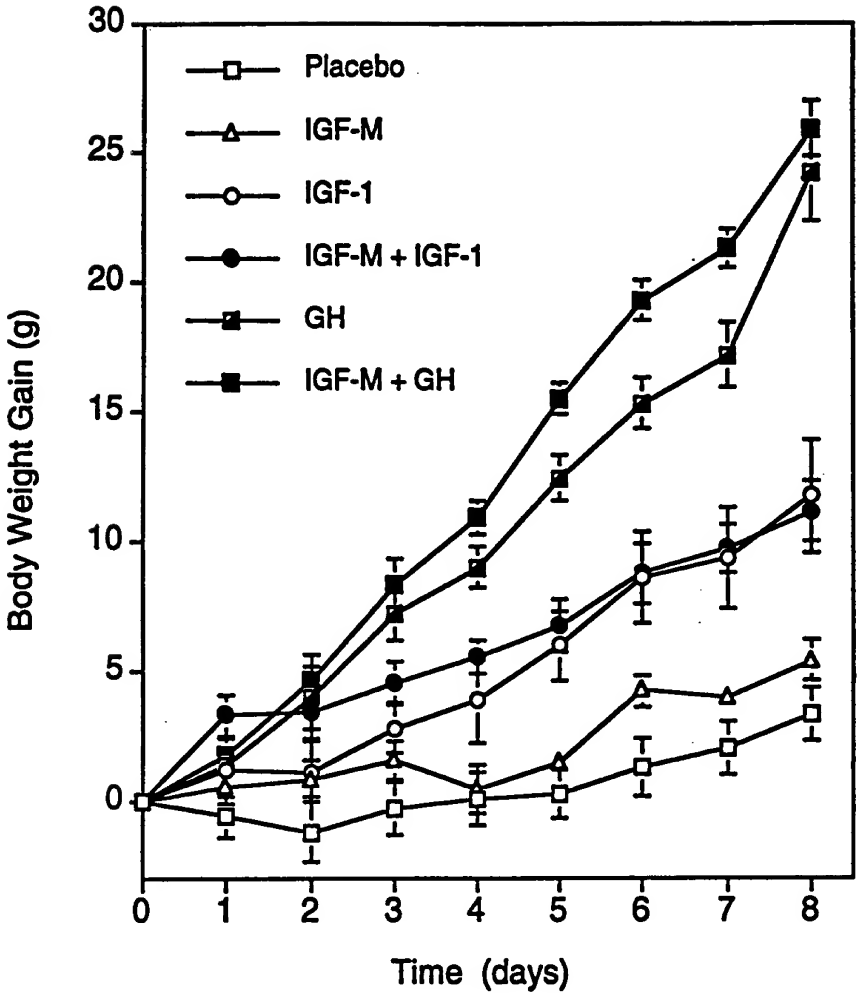

Placebo
IGF-M
IGF-1
IGF-M + IGF-1
GH
IGF-M + GH
Body Weight Gain (g)
Time (days)
0
5
10
15
20
25
30
0
1
2
3
4
5
6
7
8

FIG. 19

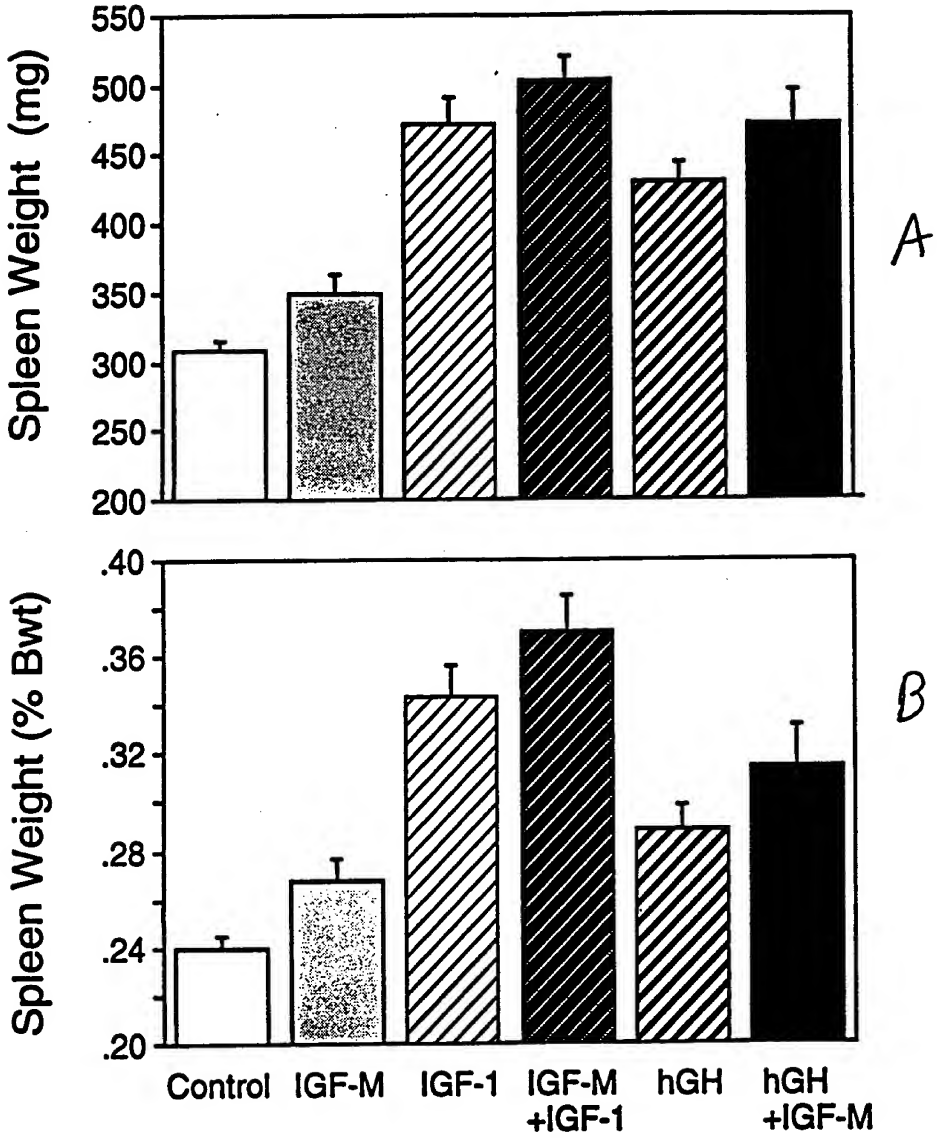

Spleen Weight (mg)
550
500
450
400
350
300
250
200
A
Spleen Weight (% Bwt)
.40
.36
.32
.28
.24
.20
B
Control
IGF-M
IGF-1
IGF-M +IGF-1
hGH
hGH +IGF-M

FIG. 20

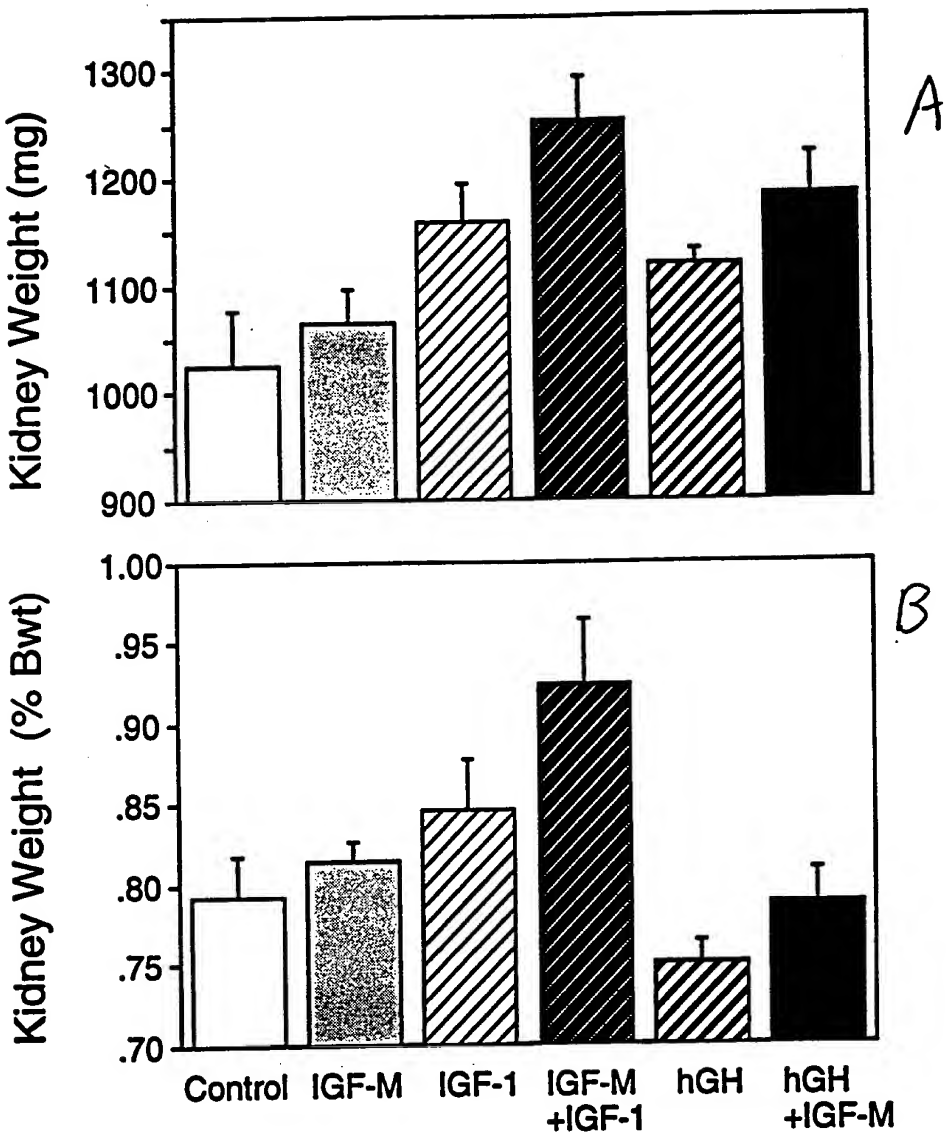

Kidney Weight (mg)
1300
1200
1100
1000
900
A
Kidney Weight (% Bwt)
1.00
.95
.90
.85
.80
.75
.70
B
Control
IGF-M
IGF-1
IGF-M +IGF-1
hGH
hGH +IGF-M

FIG. 21

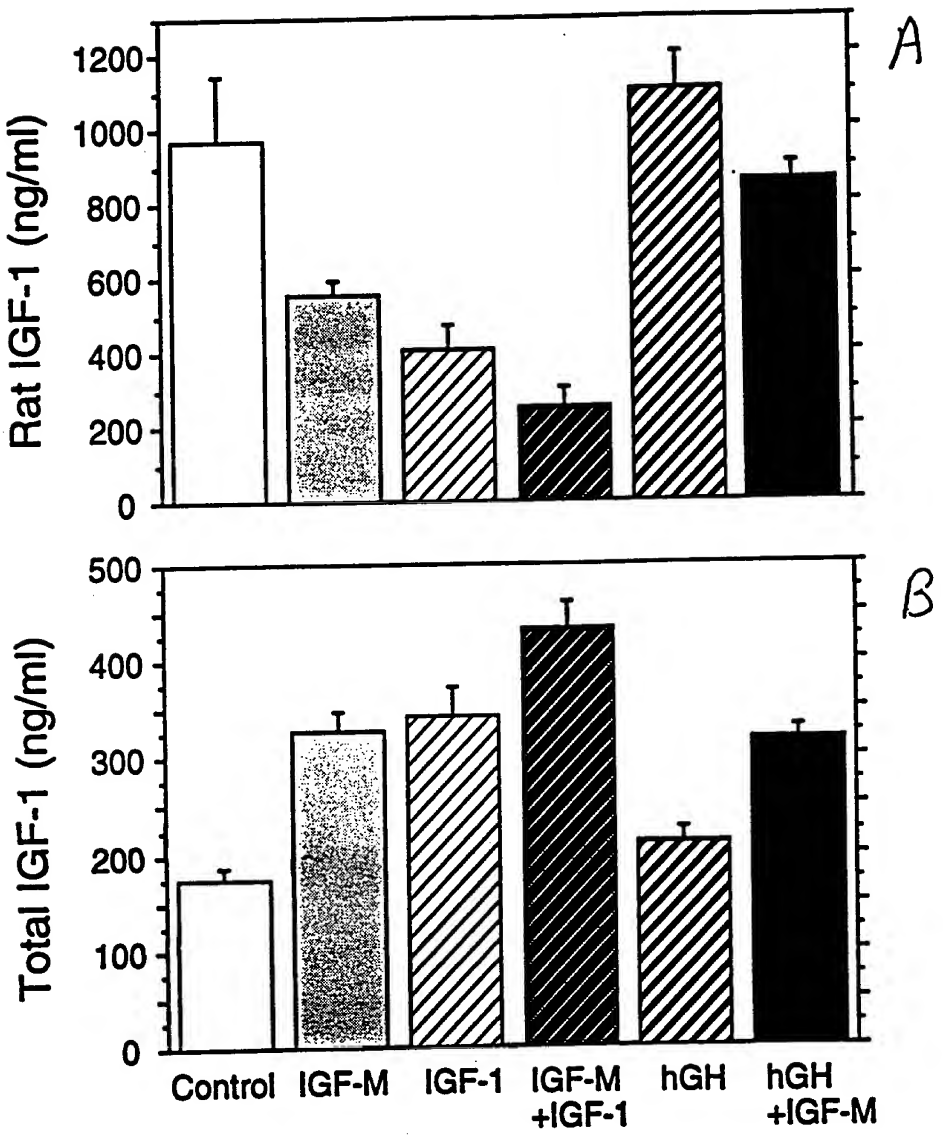

A
Rat IGF-1 (ng/ml)
1200
1000
800
600
400
200
0
B
Total IGF-1 (ng/ml)
500
400
300
200
100
0
Control
IGF-M
IGF-1
IGF-M +IGF-1
hGH
hGH +IGF-M

FIG. 22

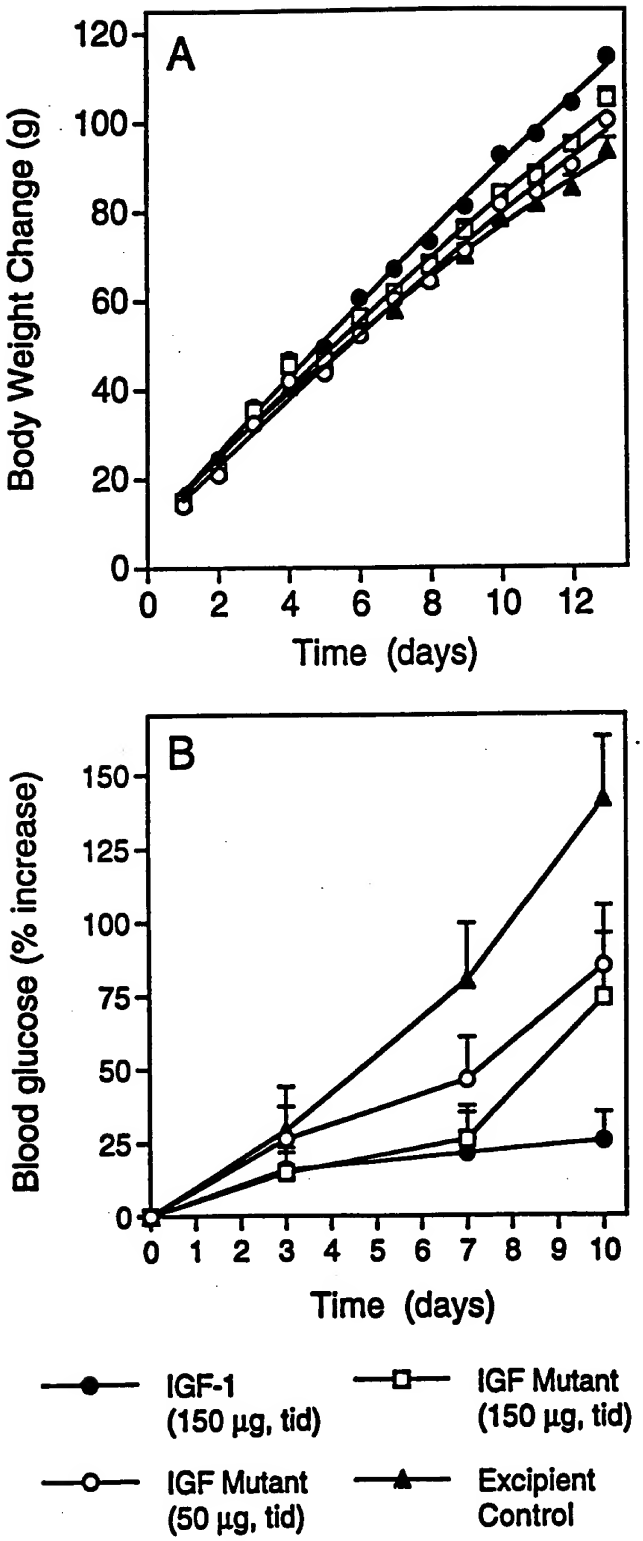

A
Body Weight Change (g)
120
100
80
60
40
20
0
0 2 4 6 8 10 12
Time (days)
B
Blood glucose (% increase)
150
125
100
75
50
25
0
0 1 2 3 4 5 6 7 8 9 10
Time (days)
IGF-1 (150 µg, tid)
IGF Mutant (150 µg, tid)
IGF Mutant (50 µg, tid)
Excipient Control

FIG. 23

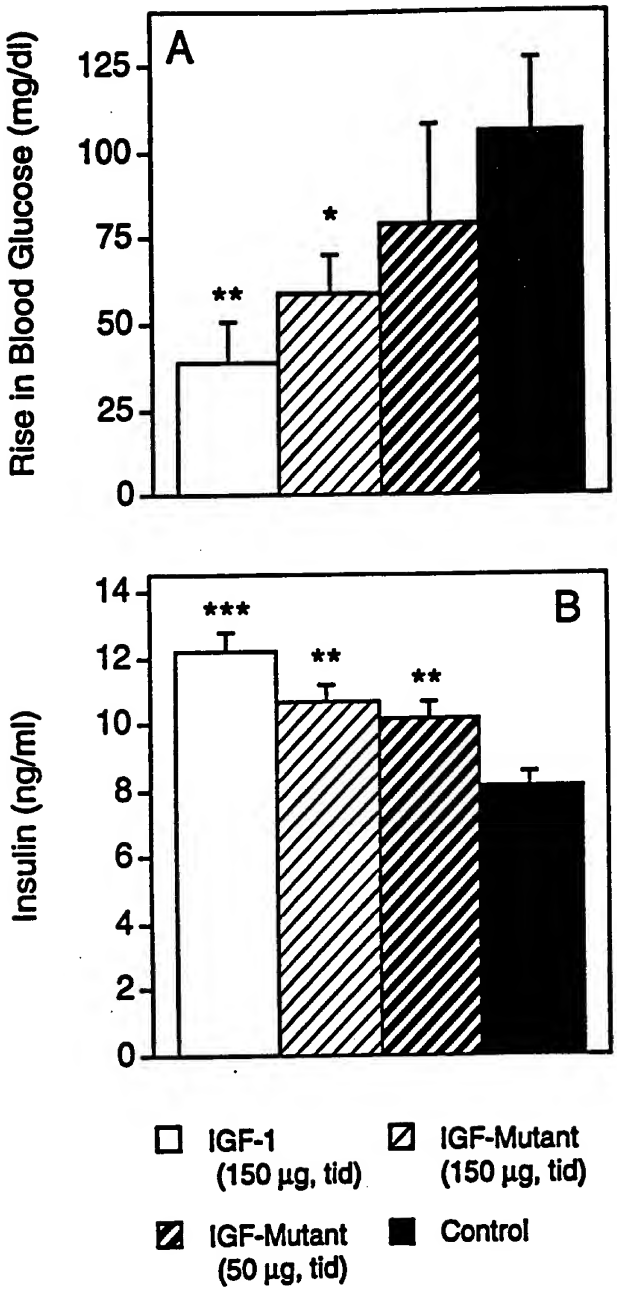

A
Rise in Blood Glucose (mg/dl)
125
100
75
50
25
0
**
*
B
Insulin (ng/ml)
14
12
10
8
6
4
2
0
***
**
**
IGF-1 (150 µg, tid)
IGF-Mutant (150 µg, tid)
IGF-Mutant (50 µg, tid)
Control

FIG. 24

plasmid t4.g8
length: 5140 (circular)

```
     1 GAATTCAACT TCTCCATACT TTGGATAAGG AAATACAGAC ATGAAAAATC TCATTGCTGA GTTGTTATTT AAGCTTGCCC AAAAAGAAGA AGAGTCGAAT
       CTTAAGTTGA AGAGGTATGA AACCTATTCC TTTATGTCTG TACTTTTTAG AGTAACGACT CAACAATAAA TTCGAACGGG TTTTTCTTCT TCTCAGCTTA

   101 GAACTGTGTG CGCAGGTAGA AGCTTTGGAG ATTATCGTCA CTGCAATGCT TCGCAATATG GCGCAAAATG ACCAACAGCG GTTGATTGAT CAGGTAGAGG
       CTTGACACAC GCGTCCATCT TCGAAACCTC TAATAGCAGT GACGTTACGA AGCGTTATAC CGCGTTTTAC TGGTTGTCGC CAACTAACTA GTCCATCTCC

   201 GGCCGCTGTA CGAGGTAAAG CCCGATGCCA GCATTCCTGA CGACGATACG GAGCTGCTGC GCGATTACGT AAAGAAGTTA TTGAAGCATC CTCGTCAGTA
       CCCGCGACAT GCTCCATTTC GGGCTACGGT CGTAAGGACT GCTGCTATGC CTCGACGACG CGCTAATGCA TTTCTTCAAT AACTTCGTAG GAGCAGTCAT

   301 AAAAGTTAAT CTTTTCAACA GCTGTCATAA AGTTGTCACG GCCGAGACTT ATAGTCGCTT TGTTTTTATT TTTTAATGTA TTTGTAACTA GTACGCAAGT
       TTTTCAATTA GAAAAGTTGT CGACAGTATT TCAACAGTGC CGGCTCTGAA TATCAGCGAA ACAAAAATAA AAAATTACAT AAACATTGAT CATGCGTTCA

   101 TCACGTAAAA AGGGTATCTA GAGGTTGAGG TGATTTTATG AAAAAGAATA TCGCATTTCT TCTTGCATCT ATGTTCGTTT TTTCTATTGC TACAAATGCC
       AGTGCATTTT TCCCATAGAT CTCCAACTCC ACTAAAATAC TTTTTCTTAT AGCGTAAAGA AGAACGTAGA TACAAGCAAA AAAGATAACG ATGTTTACGG

   501 TATGCATCTG GTACCGCCAT GGCTGATCCG AACCGTTTCC GCGGTAAAGA TCTGGCAGGT TCACCACGTG GAGGATCCGG AGGAGGCGCC GAGGGTGACG
       ATACGTAGAC CATGGCGGTA CCGACTAGGC TTGGCAAAGG CGCCATTTCT AGACCGTCCA AGTGGTCCAC CTCCTAGGCC TCCTCCGCGG CTCCCACTGC
     1       SerG lyThrAlaMe tAlaAspPro AsnArgPheA rgGlyLysAs pLeuAlaGly SerProGlyG lyGlySerGl yGlyGlyAla GluGlyAspAsp

   601 ATCCCGCAAA AGCGGCCTTT AACTCCCTGC AAGCCTCAGC GACCGAATAT ATCGGTTATG CGTGGGCGAT GGTTGTTGTC ATTGTCGGCG CAACTATCGG
       TAGGGCGTTT TCGCCGGAAA TTGAGGGACG TTCCGAGTCG CTGGCTTATA TAGCCAATAC GCACCCGCTA CCAACAACAG TAACAGCCGC GTTGATAGCC
    33   ProAlaLy sAlaAlaPhe AsnSerLeuG lnAlaSerAl aThrGluTyr IleGlyTyrA laTrpAlaMe tValValVal IleValGlyA laThrIleGly

   701 TATCAAGCTG TTTAAGAAAT TCACCTCGAA AGCAAGCTGA TAAACCGATA CAATTAAAGG CTCCTTTTGG AGCCTTTTTT TTTGGAGATT TTCAACGTGA
       ATAGTTCGAC AAATTCTTTA AGTGGAGCTT TCGTTCGACT ATTTGGCTAT GTTAATTTCC GAGGAAAACC TCGGAAAAAA AAACCTCTAA AAGTTGCACT
    66  IleLysLeu PheLysLysP heThrSerLy sAlaSer

   801 AAAAATTATT ATTCGCAATT CCTTTAGTTG TTCCTTTCTA TTCTCACTCC GCTGAAACTG TTGAAAGTTG TTTAGCAAAA CCCCATACAG AAAATTCATT
       TTTTTAATAA TAAGCGTTAA GGAAATCAAC AAGGAAAGAT AAGAGTGAGG CGACTTTGAC AACTTTCAAC AAATCGTTTT GGGGTATGTC TTTTAAGTAA

       TACTAACGTC TGGAAAGACG ACAAAACTTT AGATCGTTAC GCTAACTATG AGCGTTGTCT CTGCAATGCT ACAGGCGTTG TAGTTTGTAC TGGTGACGAA
       ATGATTGCAG ACCTTTCTGC TGTTTTGAAA TCTAGCAATG CGATTGATAC TCCCAACAGA CACCTTACGA TGTCCGCAAC ATCAAACATG ACCACTGCTT

  1001 ACTCAGTGTC TAGCTAGAGT GGCGGTGGCT CTGGTTCCGG TGATTTTGAT TATGAAAAGA TGGCAAACGC TAATAAGGGG GCTATGACCG AAAATGCCGA
       TGAGTCACAG ATCGATCTCA CCGCCACCGA GACCAAGGCC ACTAAAACTA ATACTTTTCT ACCGTTTGCG ATTATTCCCC CGATACTGGC TTTTACGGCT

  1101 TGAAAACGCG CTACAGTCTG ACGCTAAAGG CAAACTTGAT TCTGTCGCTA CTGATTACGG TGCTGCTATC GATGGTTTCA TTGGTGACGT TTCCGGCCTT
       ACTTTTGCGC GATGTCAGAC TGCGATTTCC GTTTGAACTA AGACAGCGAT GACTAATGCC ACGACGATAG CTACCAAAGT AACCACTGCA AAGGCCGGAA

  1201 GCTAATGGTA ATGGTGCTAC TGGTGATTTT GCTGGCTCTA ATTCCCAAAT CGCTCAAGTC CGTGACGGTG ATAATTCACC TTTAATGAAT AATTTCCGTC
       CGATTACCAT TACCACGATG ACCACTAAAA CGACCGAGAT TAAGGCTTTA CCGAGTTCAG CCACTGCCAC TATTAAGTGG AAATTACTTA TTAAAGGCAG

  1301 AATATTTACC TTCCCTCCCT CAATCGGTTG AATGTCGCCC TTTTGTCTTT AGCGCTGGTA AACCATATGA ATTTTCTATT GATTGTGACA AAATAAACTT
       TTATAAATGG AAGGGAGGGA GTTAGCCAAC TTACAGCGGG AAAACAGAAA TCGCGACCAT TTGGTATACT TAAAAGATAA CTAACACTCT TTTATTTGAA

  1401 ATTCCGTGGT GTCTTTCCGT TTCTTTTATA TGTTGCCACC TTTATGTATG TATTTTCTAC GTTTGCTAAC ATACTGCGTA ATAAGGAGTC TTAATCATGC
       TAAGGCACCA CAGAAACGCA AAGAAAATAT ACAACGGTGG AAATACATAC ATAAAAGATG CAAACGATTG TATGACGCAT TATTCCTCAG AATTAGTACG
```

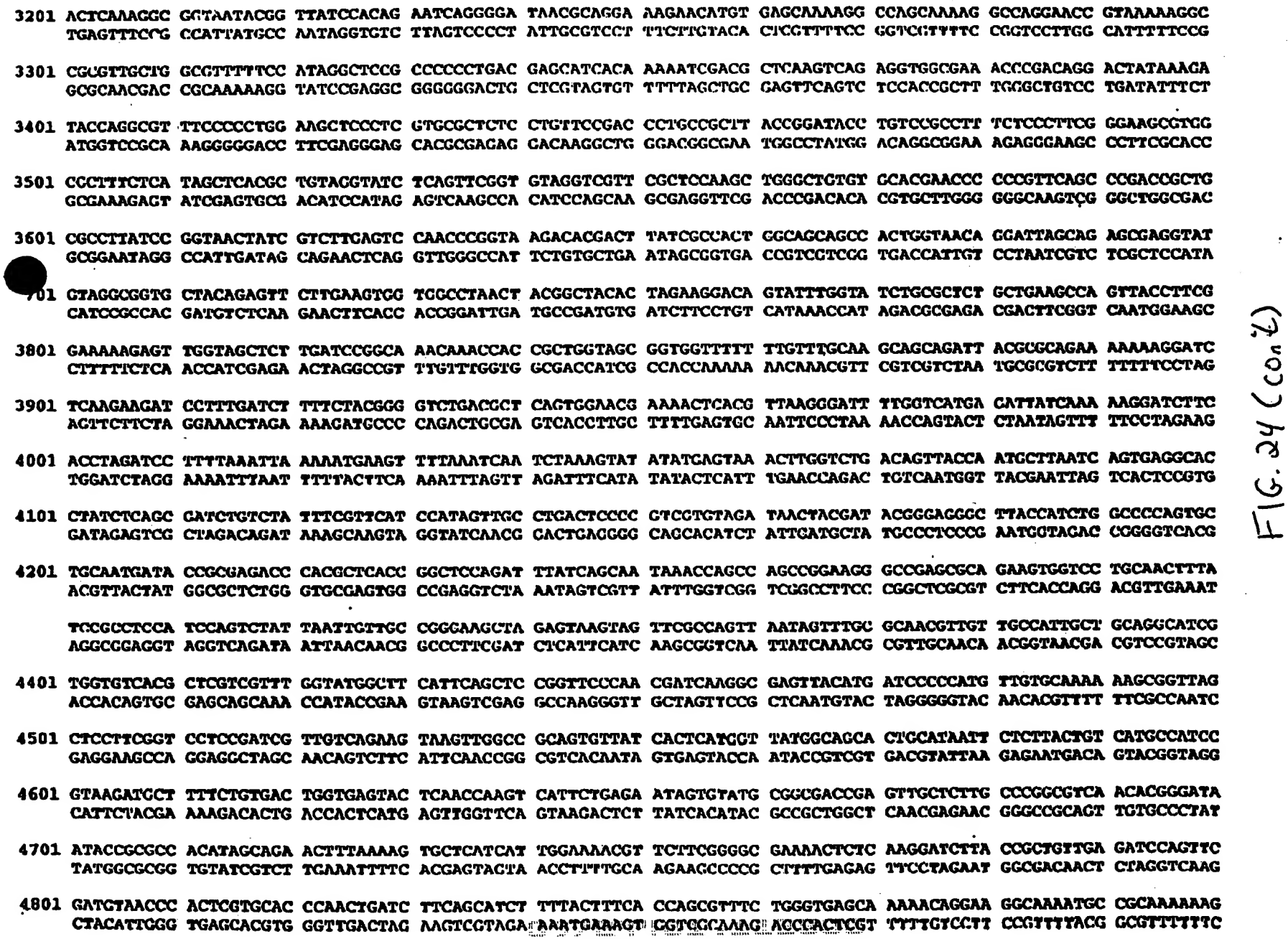


FIG. 24 (cont.)

```
4901 GGAATAAGGG CGACACGGAA ATGTTGAATA CTCATACTCT TCCTTTTTCA ATATTATTGA AGCATTTATC AGGGTTATTG TCTCATGAGC GGATACATAT
     CCTTATTCCC GCTGTGCCTT TACAACTTAT GAGTATGAGA AGGAAAAAGT TATAATAACT TCGTAAATAG TCCCAATAAC AGAGTACTCG CCTATGTATA

5001 TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCCGCG CACATTTCCC CGAAAAGTGC CACCTGACGT CTAAGAAACC ATTATTATCA TGACATTAAC
     AACTTACATA AATCTTTTTA TTTGTTTATC CCCAAGGCGC GTGTAAAGGG GCTTTTCACG GTGGACTGCA GATTCTTTGG TAATAATAGT ACTGTAATTG

5101 CTATAAAAAT AGCCGTATCA CGAGGCCCTT TCGTCTTCAA
     GATATTTTTA TCCGCATAGT GCTCCGGGAA AGCAGAAGTT
```

FIG. 24 (cont)

FIG. 25

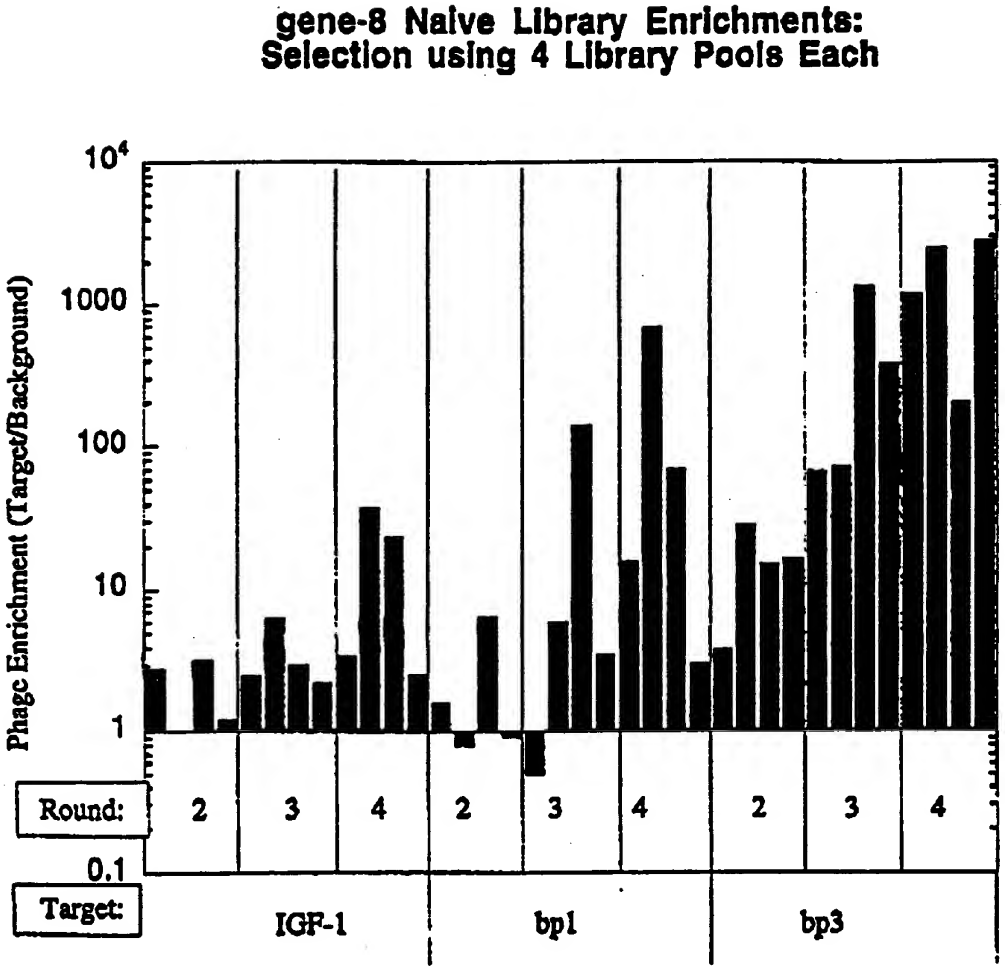

gene-8 Naive Library Enrichments:
Selection using 4 Library Pools Each
Phage Enrichment (Target/Background)
10⁴
1000
100
10
1
0.1
Round:
2
3
4
2
3
4
2
3
4
Target:
IGF-1
bp1
bp3

FIG. 26

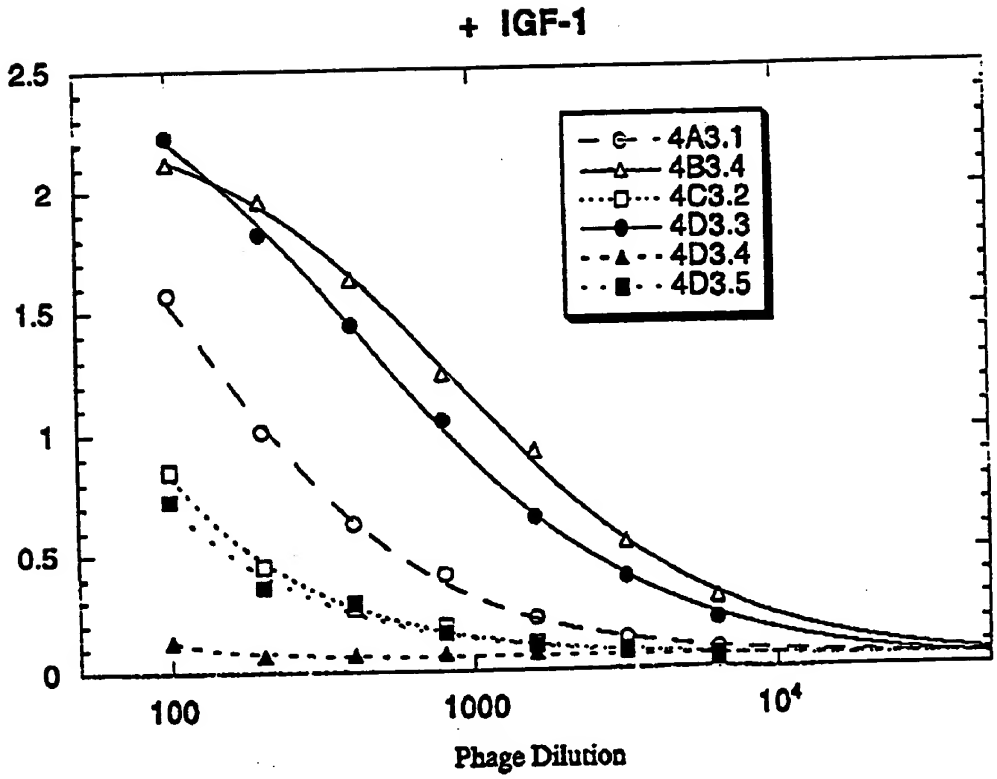

+ IGF-1
Phage bound (OD 492)
2.5
2
1.5
1
0.5
0
100
1000
10⁴
Phage Dilution
4A3.1
4B3.4
4C3.2
4D3.3
4D3.4
4D3.5

FIG. 27

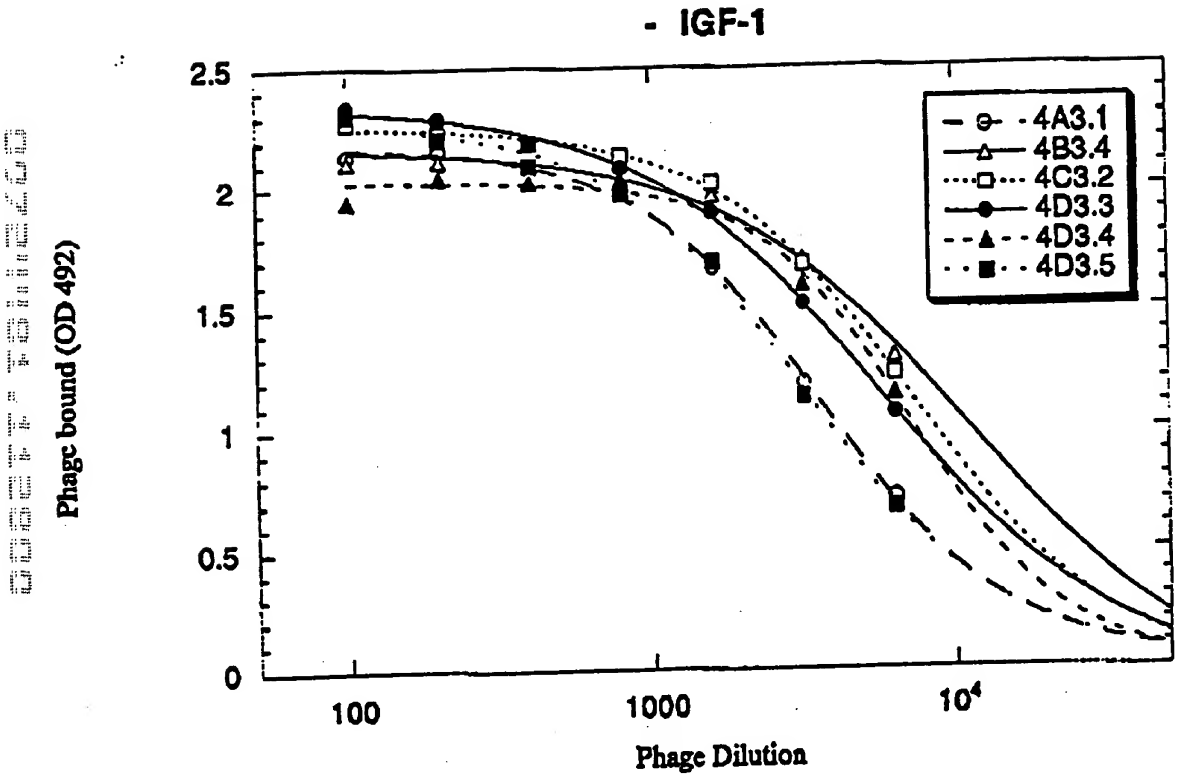

- IGF-1
2.5
2
1.5
1
0.5
0
Phage bound (OD 492)
100
1000
10⁴
Phage Dilution
4A3.1
4B3.4
4C3.2
4D3.3
4D3.4
4D3.5

FIG. 28

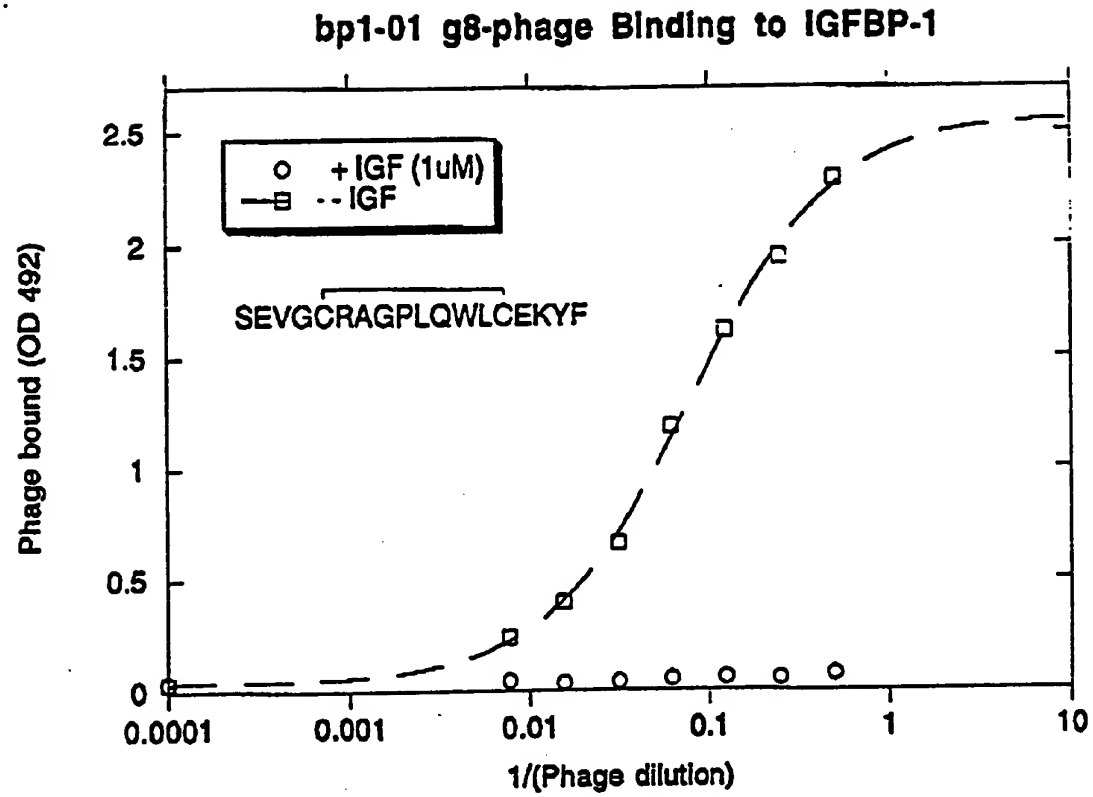

bp1-01 g8-phage Binding to IGFBP-1
+IGF (1uM)
-IGF
SEVGCRAGPLQWLCEKYF
Phage bound (OD 492)
2.5
2
1.5
1
0.5
0
0.0001
0.001
0.01
0.1
1
10
1/(Phage dilution)

FIG. 29

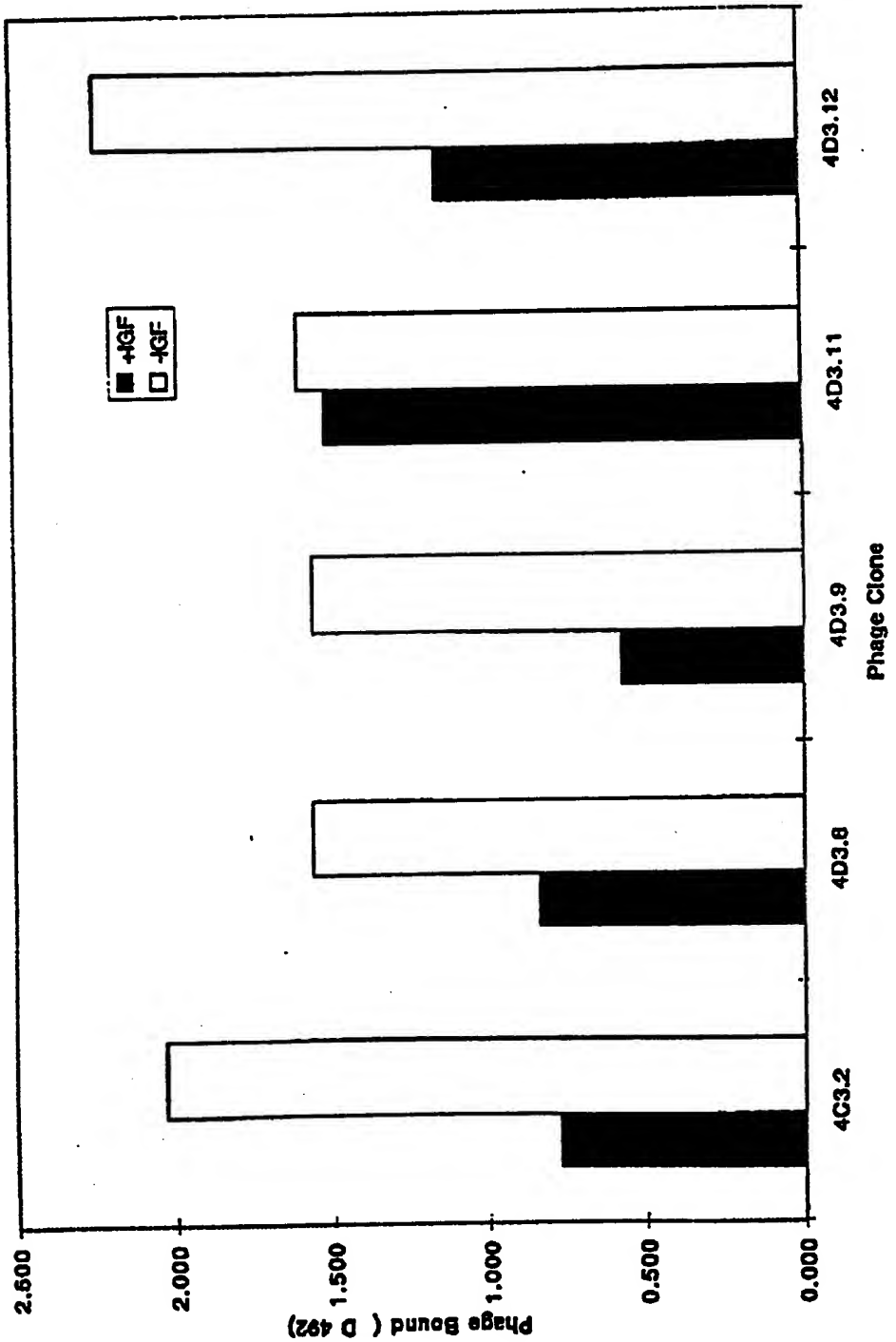

(A) PHAGE BLOCKING ASSAY
+IGF
-IGF
Phage Bound (D 492)
2.500
2.000
1.500
1.000
0.500
0.000
4C3.2
4D3.8
4D3.9
4D3.11
4D3.12
Phage Clone

FIG. 30

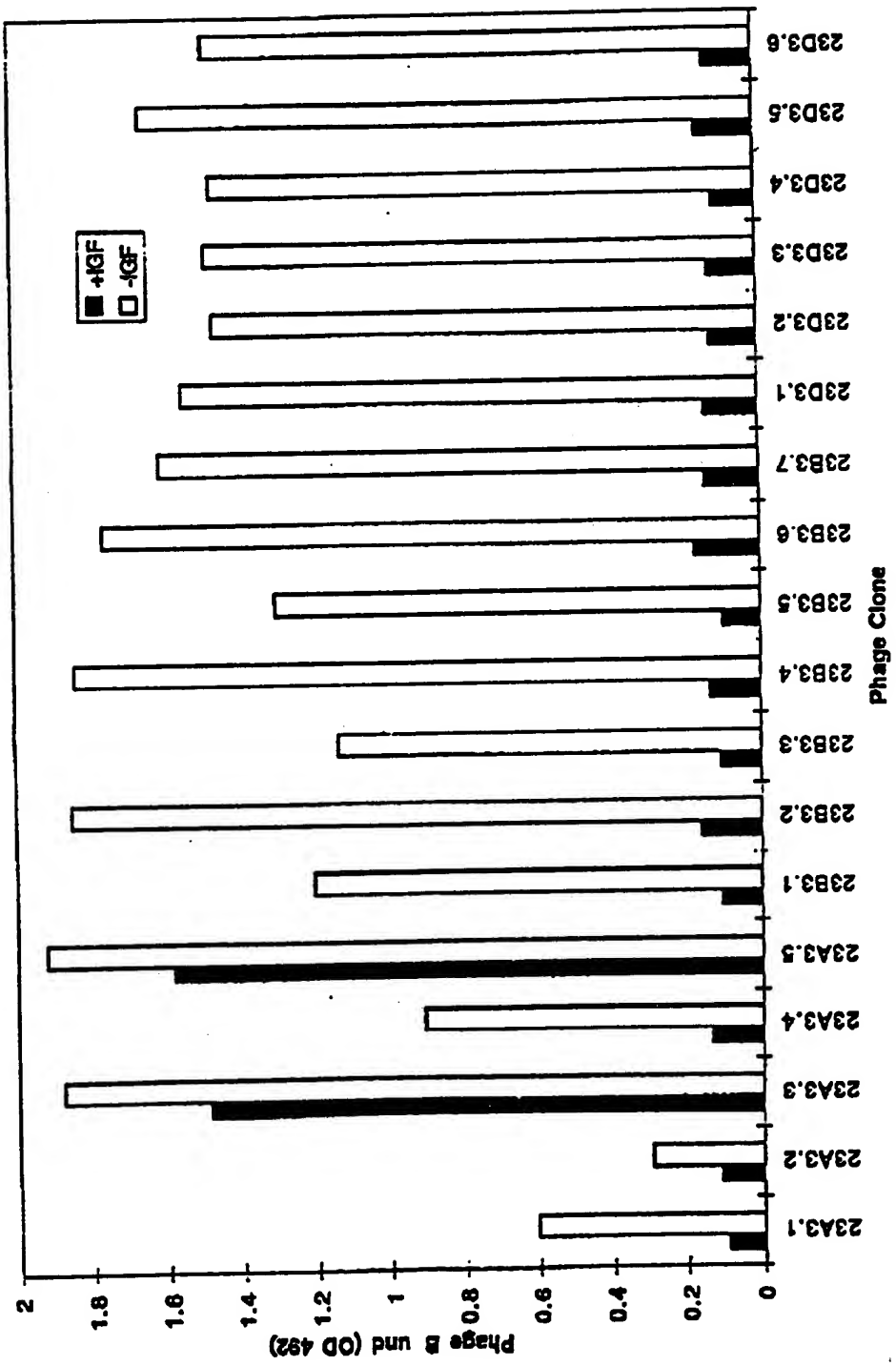

(B) PHAGE IGF BLOCKING ASSAY
Phage Clone
Phage B ind (OD 492)
+IGF
-IGF
23A3.1
23A3.2
23A3.3
23A3.4
23A3.5
23B3.1
23B3.2
23B3.3
23B3.4
23B3.5
23B3.6
23B3.7
23D3.1
23D3.2
23D3.3
23D3.4
23D3.5
23D3.6
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2

FIG. 31

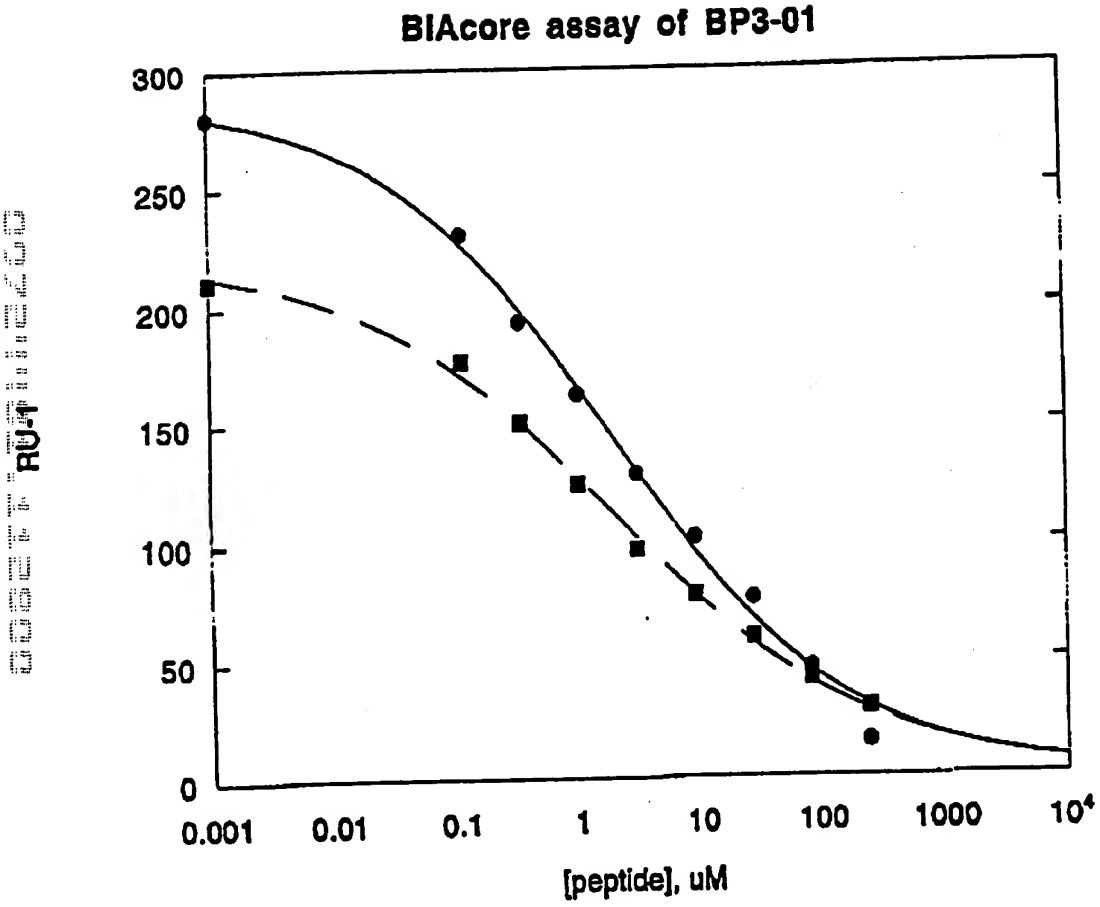

BIAcore assay of BP3-01
RU
300
250
200
150
100
50
0
0.001
0.01
0.1
1
10
100
1000
10⁴
[peptide], uM

FIG. 32

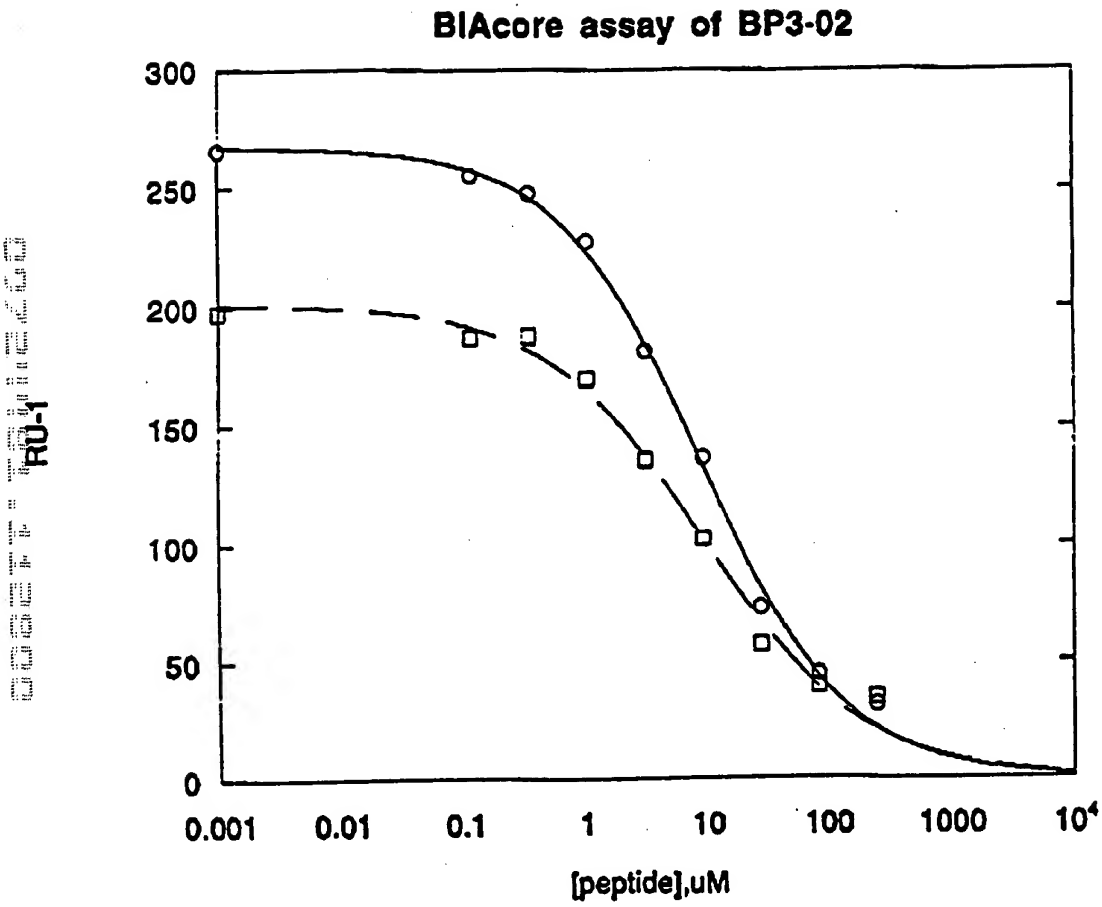

BIAcore assay of BP3-02
RU-1
300
250
200
150
100
50
0
0.001
0.01
0.1
1
10
100
1000
10⁴
[peptide],uM

FIG. 33

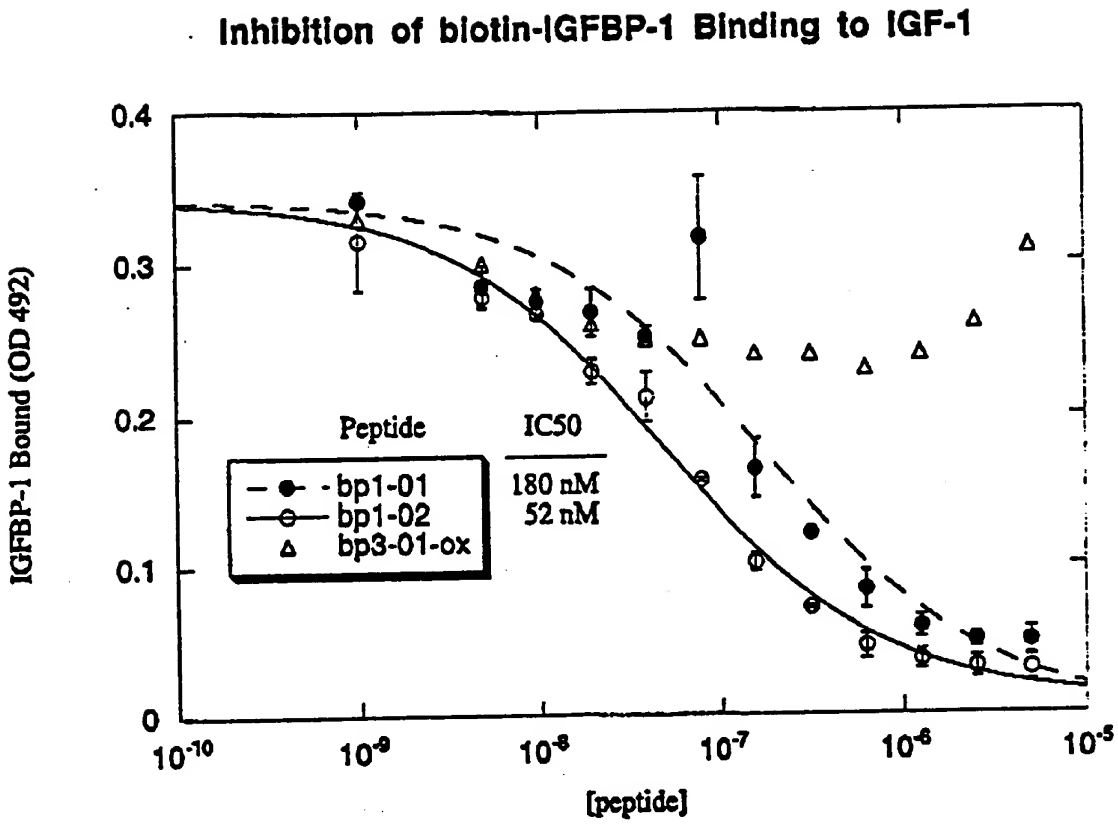

Inhibition of biotin-IGFBP-1 Binding to IGF-1
IGFBP-1 Bound (OD 492)
0.4
0.3
0.2
0.1
0
Peptide
IC50
bp1-01
180 nM
bp1-02
52 nM
bp3-01-ox
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
[peptide]

FIG. 34

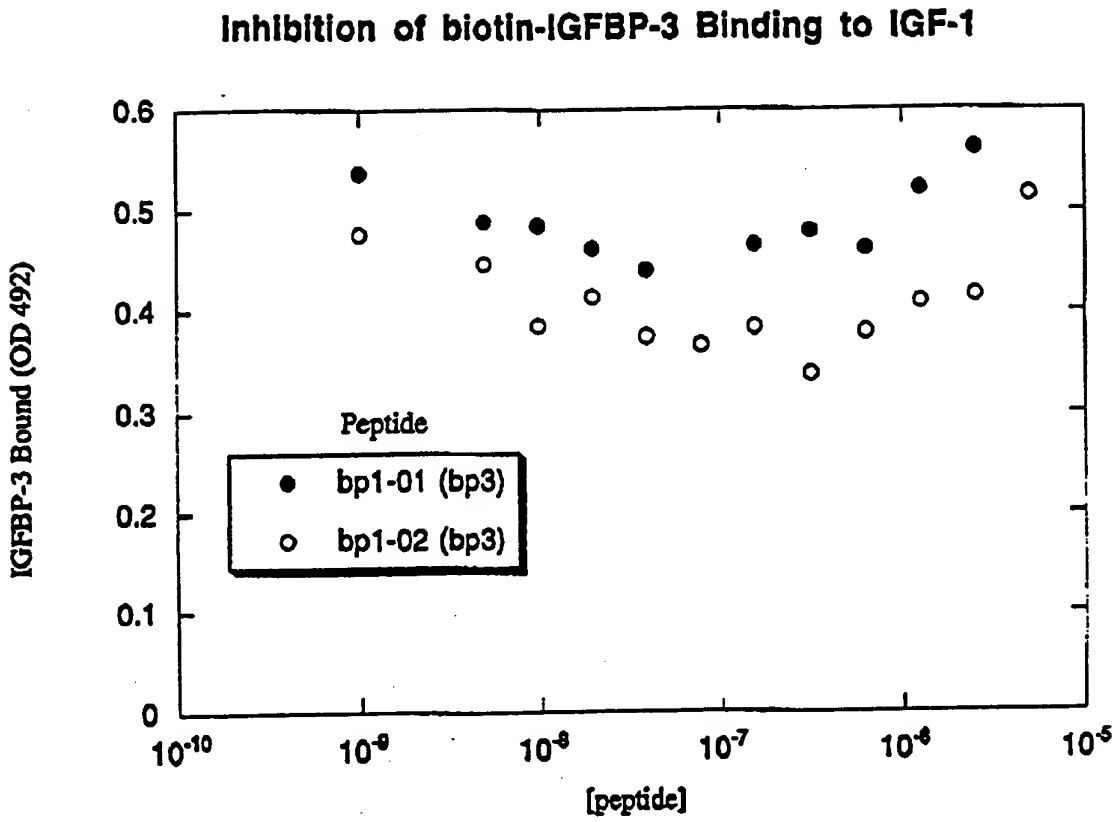

Inhibition of biotin-IGFBP-3 Binding to IGF-1
IGFBP-3 Bound (OD 492)
0.6
0.5
0.4
0.3
0.2
0.1
0
Peptide
bp1-01 (bp3)
bp1-02 (bp3)
10-10
10-9
10-8
10-7
10-6
10-5
[peptide]

FIG. 35

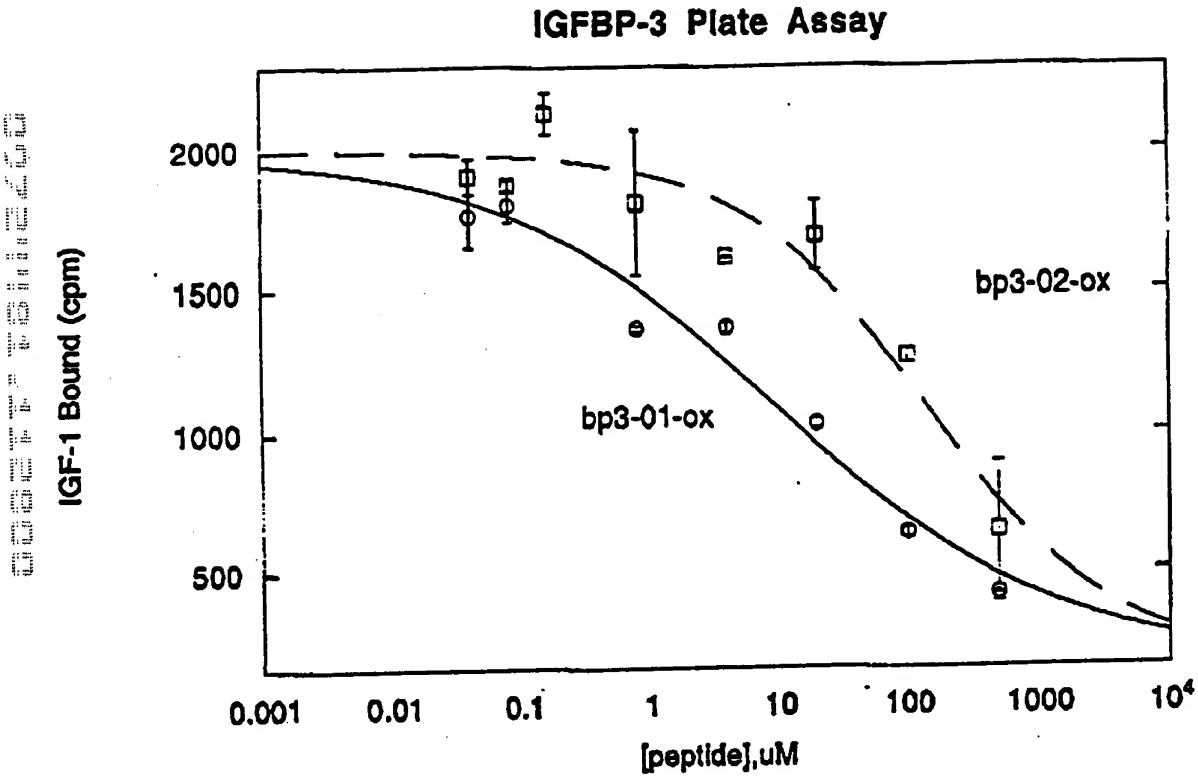

IGFBP-3 Plate Assay
IGF-1 Bound (cpm)
2000
1500
1000
500
0.001
0.01
0.1
1
10
100
1000
10⁴
[peptide],uM
bp3-02-ox
bp3-01-ox

FIG. 36

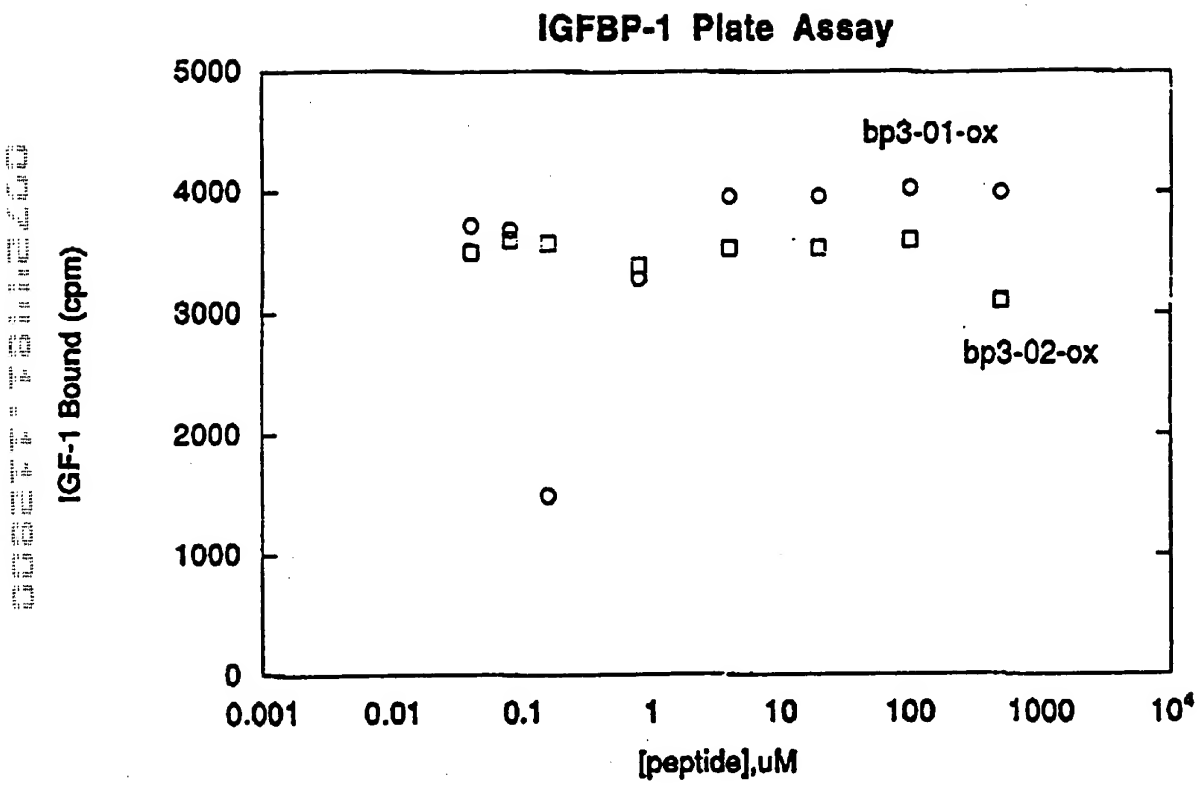

IGFBP-1 Plate Assay
IGF-1 Bound (cpm)
5000
4000
3000
2000
1000
0
0.001
0.01
0.1
1
10
100
1000
10⁴
[peptide],uM
bp3-01-ox
bp3-02-ox

FIG. 37

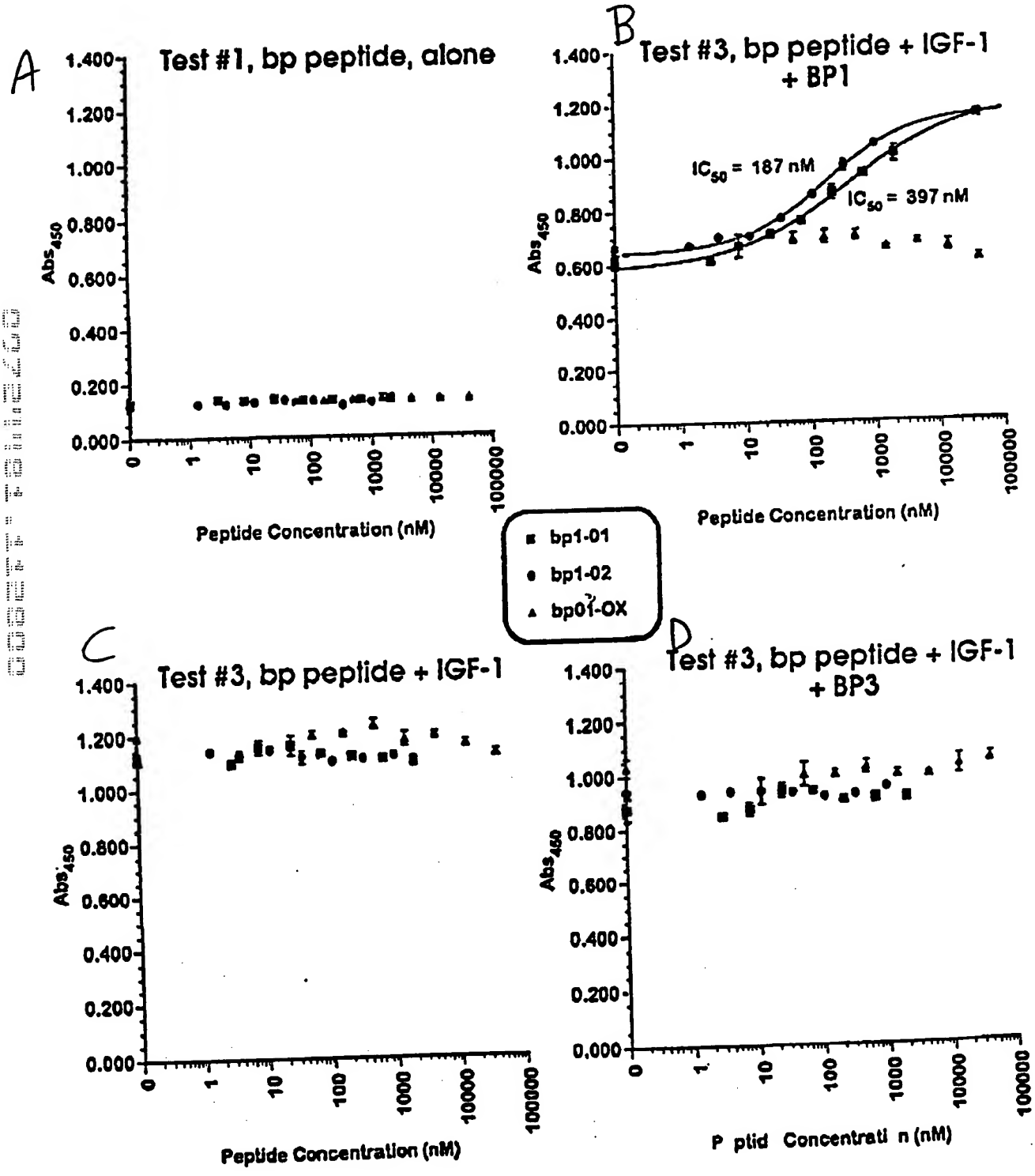

A
Test #1, bp peptide, alone
Abs450
Peptide Concentration (nM)
B
Test #3, bp peptide + IGF-1 + BP1
IC50 = 187 nM
IC50 = 397 nM
Abs450
Peptide Concentration (nM)
bp1-01
bp1-02
bp01-OX
C
Test #3, bp peptide + IGF-1
Abs450
Peptide Concentration (nM)
D
Test #3, bp peptide + IGF-1 + BP3
Abs450
Peptide Concentration (nM)

FIG. 38

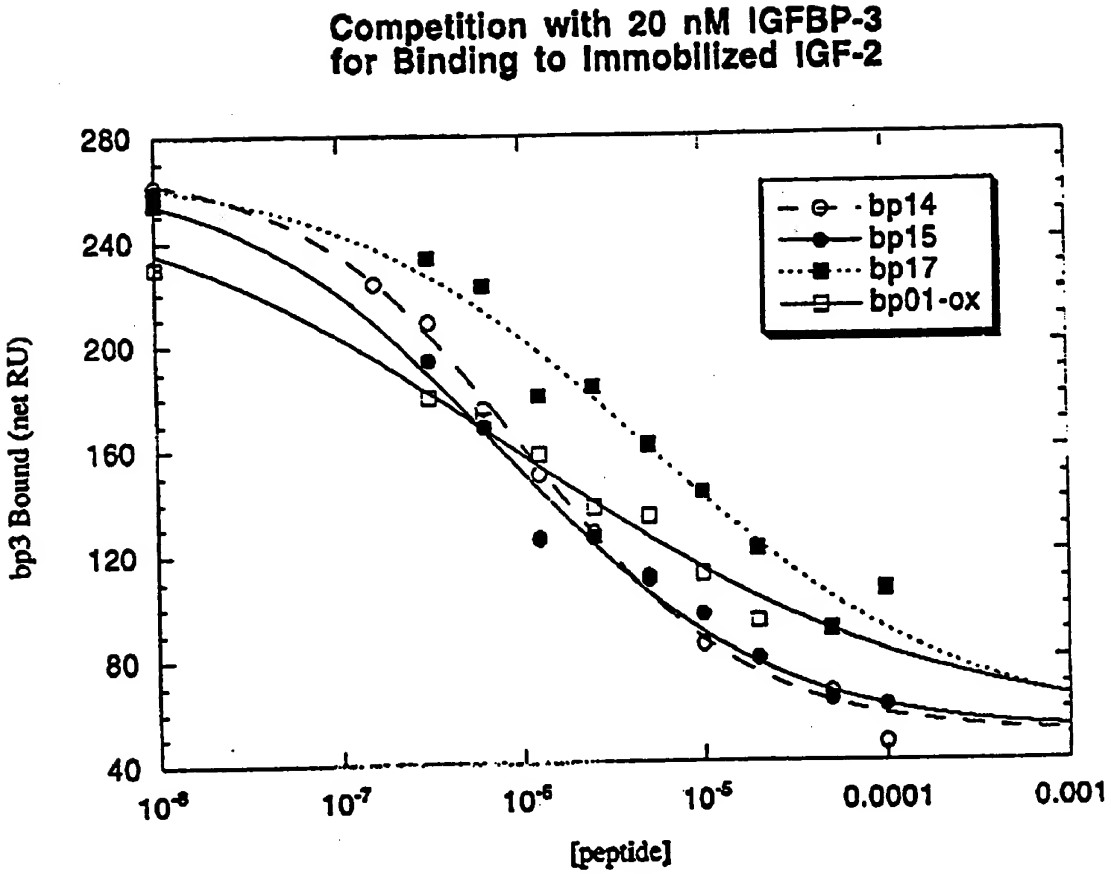

Competition with 20 nM IGFBP-3
for Binding to Immobilized IGF-2
bp3 Bound (net RU)
280
240
200
160
120
80
40
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
0.0001
0.001
[peptide]
bp14
bp15
bp17
bp01-ox

FIG. 39

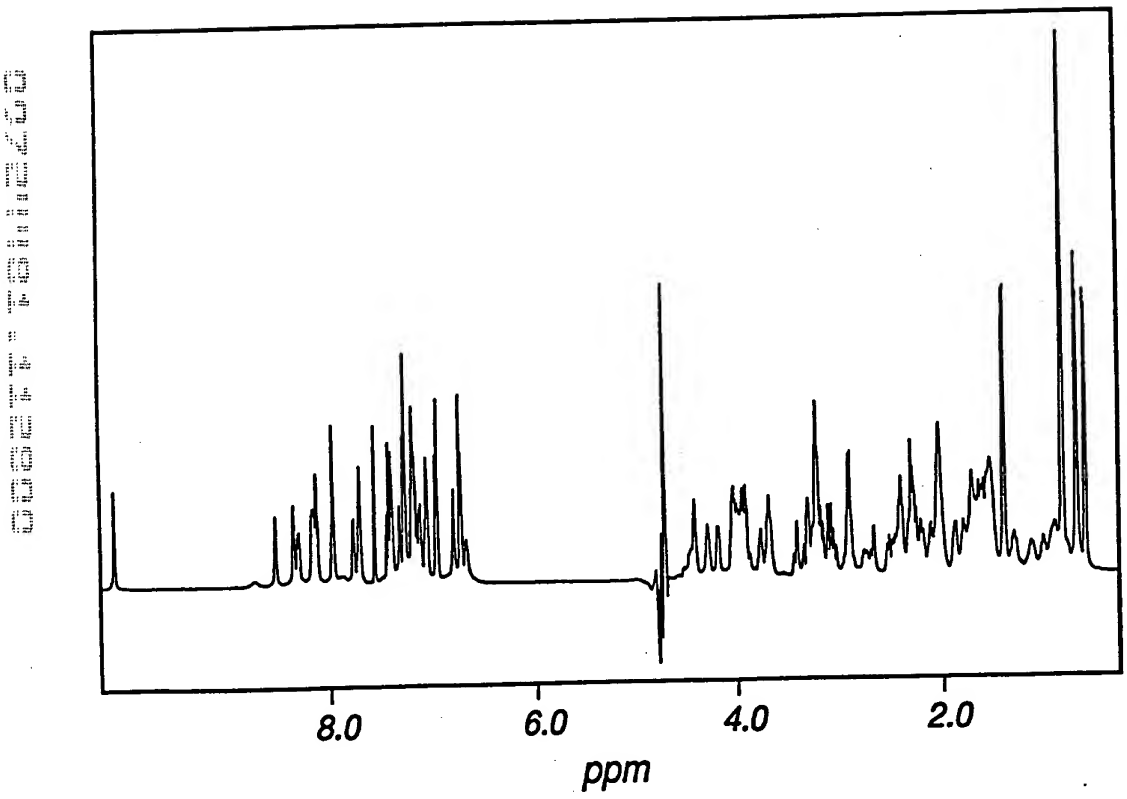


FIG. 40

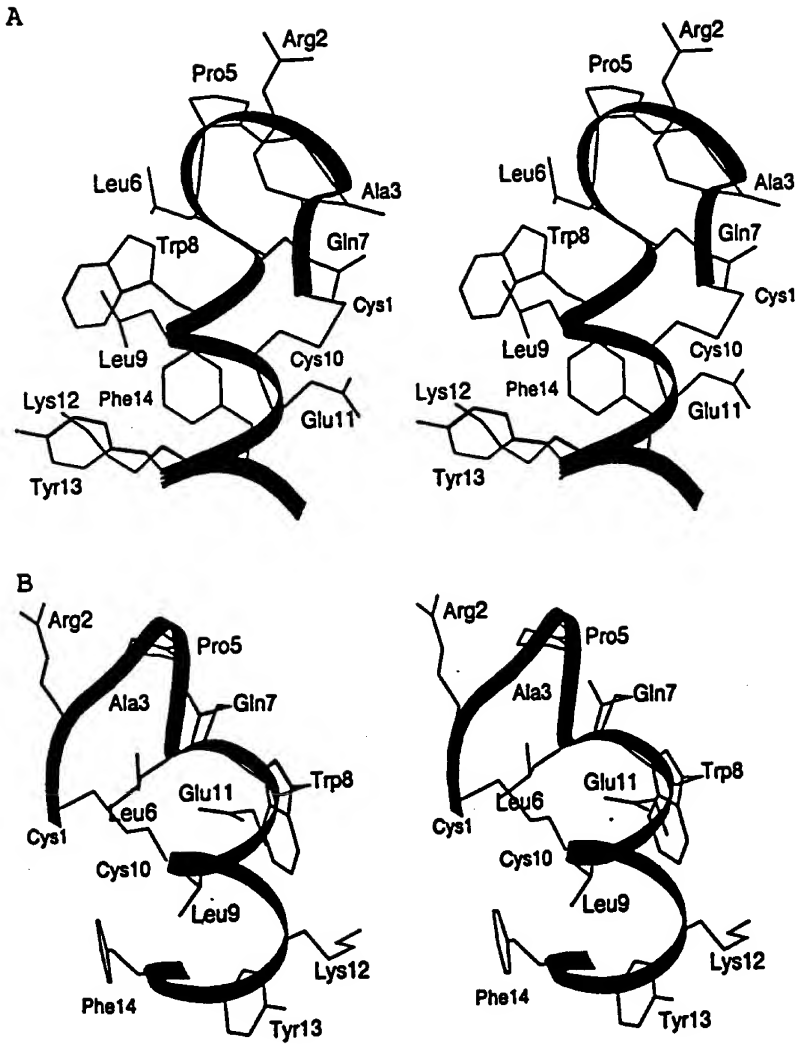

A
Arg2
Pro5
Leu6
Ala3
Trp8
Gln7
Cys1
Leu9
Cys10
Lys12
Phe14
Glu11
Tyr13
Arg2
Pro5
Leu6
Ala3
Trp8
Gln7
Cys1
Leu9
Cys10
Lys12
Phe14
Glu11
Tyr13
B
Arg2
Pro5
Ala3
Gln7
Glu11
Trp8
Leu6
Cys1
Cys10
Leu9
Lys12
Phe14
Tyr13
Arg2
Pro5
Ala3
Gln7
Glu11
Trp8
Leu6
Cys1
Cys10
Leu9
Lys12
Phe14
Tyr13

FIG. 41

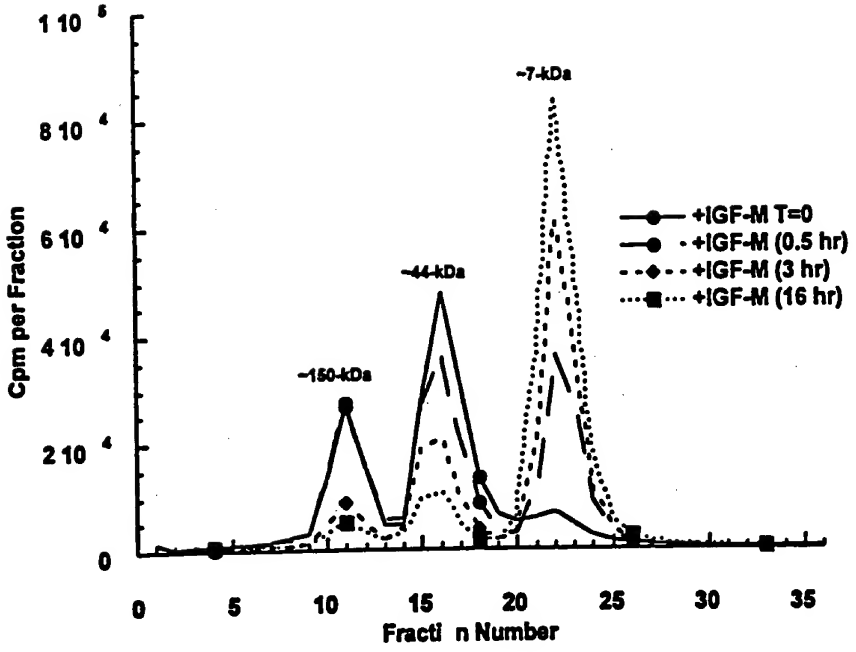

1 10 5
8 10 4
6 10 4
4 10 4
2 10 4
0
Cpm per Fraction
~150-kDa
~44-kDa
~7-kDa
+IGF-M T=0
+IGF-M (0.5 hr)
+IGF-M (3 hr)
+IGF-M (16 hr)
0
5
10
15
20
25
30
35
Fracti n Number

FIG. 42

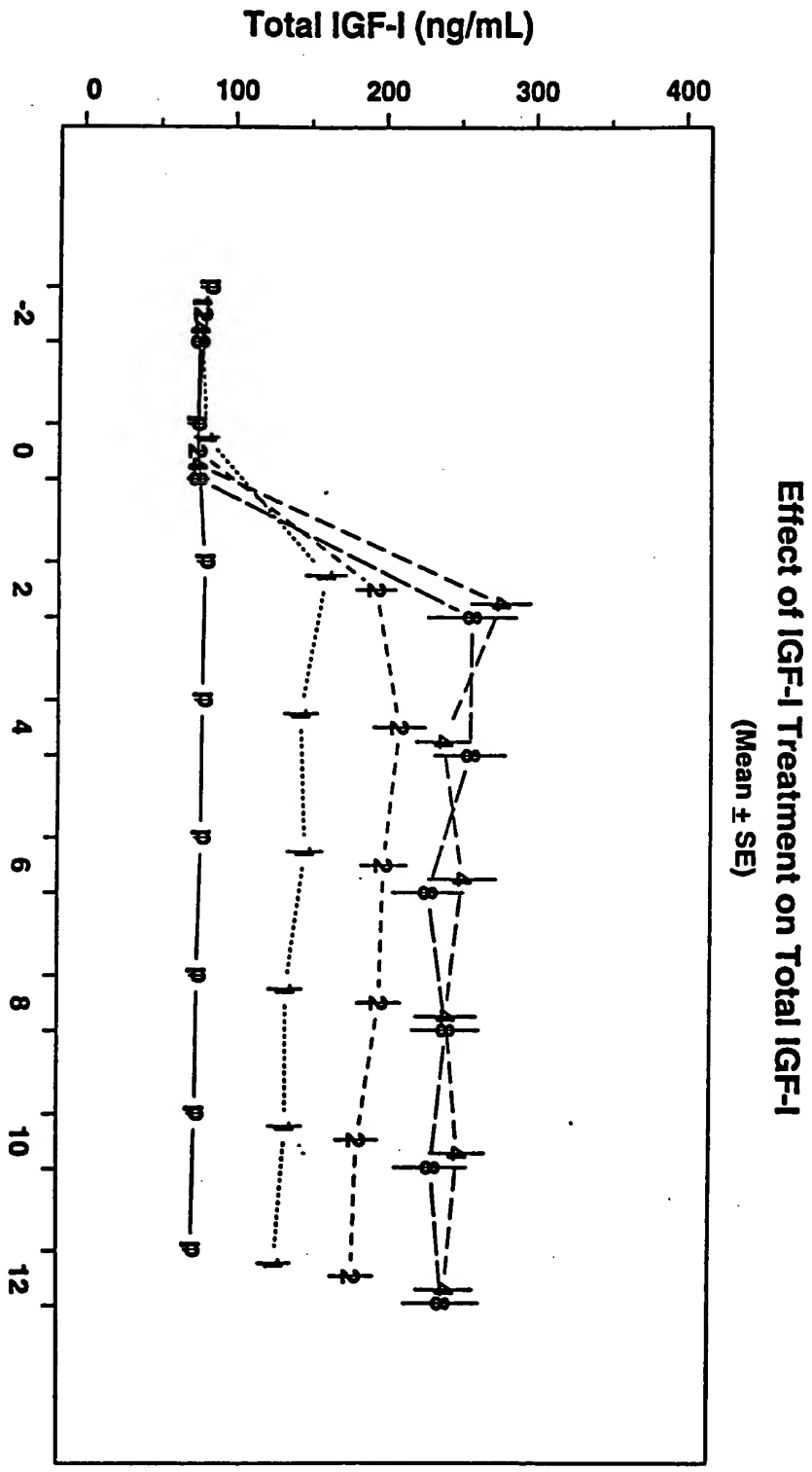

Effect of IGF-I Treatment on Total IGF-I
(Mean ± SE)
Total IGF-I (ng/mL)
0
100
200
300
400
-2
0
2
4
6
8
10
12
Treatment Visits (Week)
P1240
P1240
P

FIG. 43

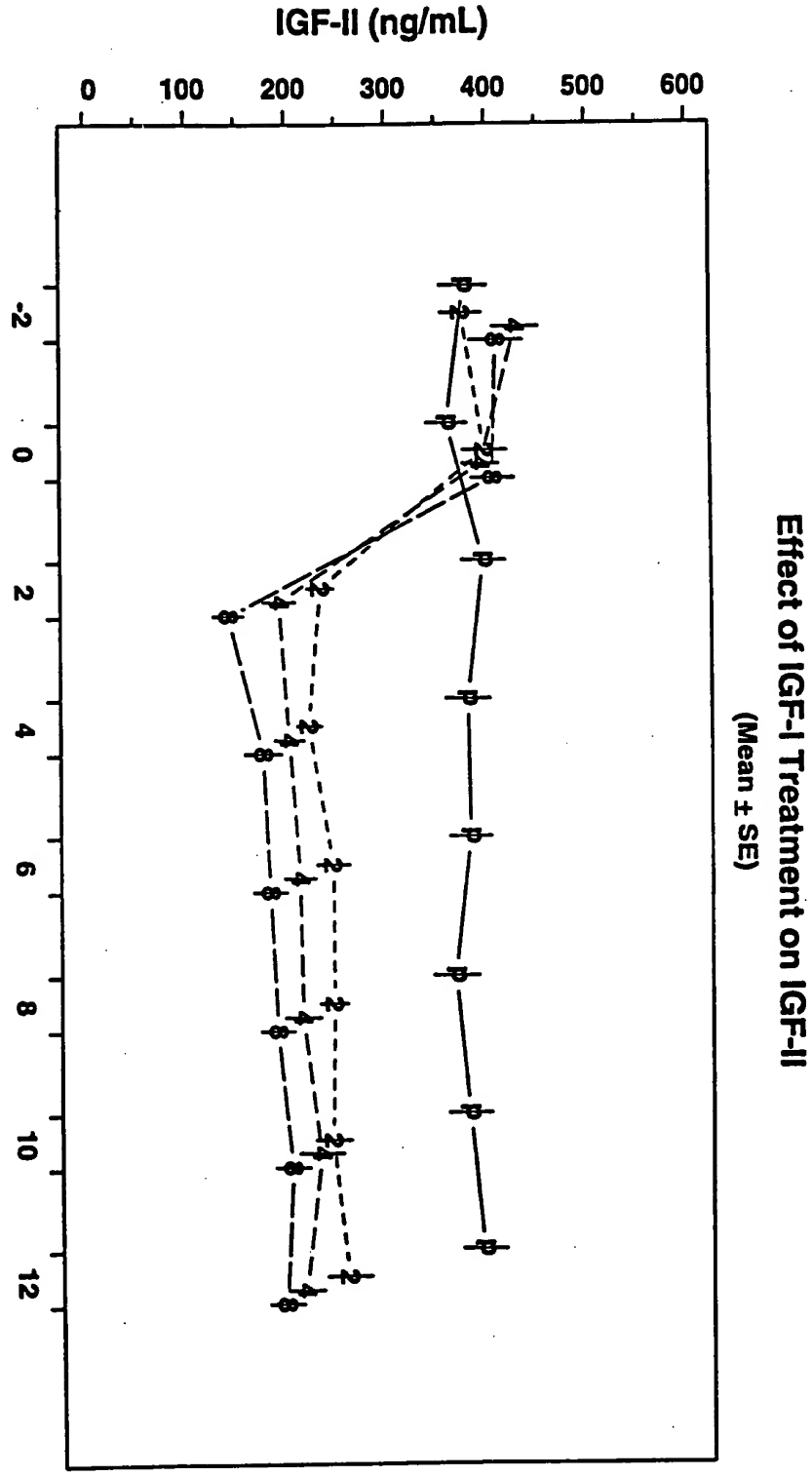
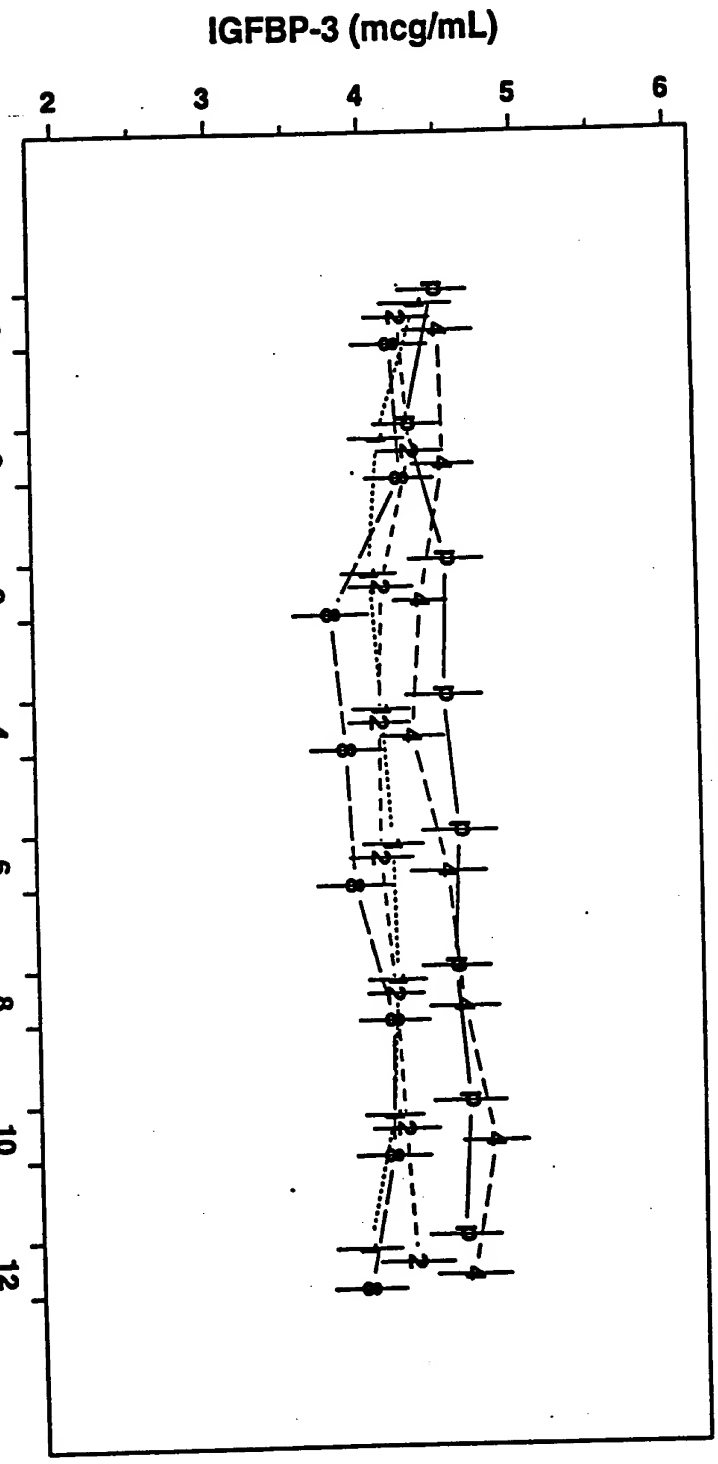

IGF-II (ng/mL)
0
100
200
300
400
500
600
-2
0
2
4
6
8
10
12
Treatment Visits (Week)
Effect of IGF-I Treatment on IGF-II
(Mean ± SE)

FIG. 44


IGFBP-3 (mcg/mL)
2
3
4
5
6
-2
0
2
4
6
8
10
12
Treatment Visits (Week)
Effect of IGF-I Treatment on IGFBP-3
(Mean ± SE)